Biochemistry. 1985 Jan 29;24(3):813-6. Related Articles, Links

Chemical synthesis of peptide fragments of the hormone-specific beta-subunit of human follicle-stimulating hormone.

Saxena BB, Rathnam P.

In order to determine the specific antigenic determinants of human follicle-stimulating hormone (hFSH), hFSH-beta peptides with amino acid residues 33-49 (V2), 95-118 (V3), 76-118 (V3 + 1/2 C2), 1-33 (V1 + C1), 22-33 (1/2C1), and 95-107 (V3 + 1/4C2) according to the nomenclature of Stewart and Stewart [Stewart, M., & Stewart, F. (1977) J. Mol. Biol. 116, 175] as well as additional peptides with the residues 93-107, 91-107, 89-107, 87-107, and 85-107 were chemically synthesized. The peptides were examined in radioimmunoassay systems of FSH, luteinizing hormone (LH), or human chorionic gonadotropin (hCG). V3 + 1/2C2 and V1 + C1 showed immunological activity, whereas the other peptides did not. Antibodies were raised in rabbits against these peptides and examined for specific binding with hFSH, LH, thyroid-stimulating hormone (TSH), and hCG. V3 + 1/2C2 as well as V1 + C1 produced antisera, which specifically bound hFSH, hLH, and hTSH, indicating that the amino acid sequences contained in hFSH-beta peptides V3 + 1/2C2 and V1 + C1 share common antigenic sites with hLH and hTSH. Antisera were produced in rabbits against hFSH-beta, against reduced and S-aminoethylated hFSH-beta (AE-FSH-beta), and against AE-FSH-beta coupled to hemocyanin. Reduced and S-aminoethylated beta-subunit of FSH-beta coupled with hemocyanin produced antisera in rabbits that specifically bound only hFSH and not hLH, hTSH, or hCG.

PMID: 2581605 [PubMed - indexed for MEDLINE]

: Mol Cell Endocrinol. 1996 Dec 20;125(1-2):33-43. Related Articles, Links

Immunochemical mapping of gonadotropins.

Berger P, Bidart JM, Delves PS, Dirnhofer S, Hoermann R, Isaacs N, Jackson A, Klonisch T, Lapthorn A, Lund T, Mann K, Roitt I, Schwarz S, Wick G.

Institute for Biomedical Aging Research, Austrian Academy of Sciences, Innsbruck, Austria. Bioage-c511@uibk.ac.at

As a glycoprotein hormone, human chorionic gonadotropic (hCG) is not a single molecular entity but this term rather comprises an array of molecular variants such as hCG, hCG beta, hCGn, hCG beta n, hCG beta cf, -CTPhCG, hCG beta CTP, deglyhCG, asialohCG, hCGav and the closely related molecules hLH, hLH beta and hLH beta ef. The advent of monoclonal antibodies (MCA), the availability of ultrasensitive detection systems and the recent determination of the crystal structure of hCG, made it possible to design special purpose diagnostic and clinical research immunoassays for hCG-like molecules. For more than a decade we and others have tried to refine epitope maps for hCG and related molecules by means of a large panel of MCA, naturally occurring metabolic variants of hCG (hCGn, hCG beta, hCG alpha, hCG beta cf, hCG beta CTP), homologous hormones and subunits of various species (e.g. hLH, hLH beta, hFSH, hTSH, oLH, rLH beta), chemically modified molecules (deglyhCG, asialohCG, tryptic and chymotryptic hCG beta and hCG alpha fragments) and synthetic peptides (octapeptides and longer). It appeared that all epitopes on molecular hCG-variants recognized by our MCA are determined by the protein backbone. Except for the two major epitopes on hCG beta CTP and parts of two antigenic domains on hCG alpha, epitopes on hCG-derived molecules are determined by the tertiary and quarternary structure. Operationally useful descriptive epitope maps were designed including information on assay suitability of antigenic determinants. On this basis we established ultrasensitive time-resolved fluoroimmuno-assays for hCG, hCG and hCGn, hCG beta and hCG beta n and hCG beta cf, hCG alpha and additional assays recognizing different spectra of hCG-variants. Such assay have been applied by us and others to the detection of pregnancy, early pregnancy loss, choriocarcinoma, testicular cancer, other cancers and prenatal diagnosis. However, as the molecular structure of many epitopes utilized in immunoassays of different laboratories was not resolved, comparability of results was not satisfactory. Consequently, attempts were made to compare schematic epitope maps from different research institutions. The situation has been much improved by solving the three-dimensional (3D) structure of hCG. It has been shown that hCG is a member of the structural superfamily of cystine knot growth factors like NGF, PDGF-B and TGF-beta. Each of its subunits is stabilized in its topology by three disulfide bonds forming a cystine knot. Moreover, it turned out that the disulfide bridges in their majority have previously been wrongly assigned. Computer molecular modeling of crystallographic coordinates of hCG and subsequent selective combined--PCR-based and immunological--mutational analyses of hCG beta expressed via the transmembrane region of a MHC molecule made it possible to more precisely localize epitopes on hCG-derived molecules. Although the entire surface of hCG has to be regarded as potentially immunogenic there seems to be hot spots where epitopes are clustered in antigenic domains. These are located on the first and third loops protuding from the cystine knots of both subunits and are possibly centered around the knot itself. Ultimate answers on epitope localizations will be given by the crystal structure determination of hCG complexed with different Fabs.

Publication Types:
- Review

Mol Cell Endocrinol. 1994 May;101(1-2):11-20. Related Articles, Links

Immunochemical mapping of human lutropin: I. Delineation of a conformational antigenic determinant.

Robert P, Troalen F, Bellet D, Bousfield GR, Bidart JM.

Service d'Immunologie Moleculaire, Institut Gustave-Roussy, Villejuif, France.

Lutropin (LH), follitropin (FSH), thyrotropin (TSH) and choriogonadotropin (CG) are assembled of two non-covalently alpha (alpha) and beta (beta) subunits. We studied the discontinuous antigenic regions recognized by a monoclonal anti-hLH antibody designated as LH05 which binds to hLH, hCG and hTSH and does not cross-react with either the free subunits or hFSH. LH05 and an antibody designated HT13, recognizing an epitope partly comprizing the alpha64-76 region, did not bind simultaneously to hCG. Furthermore, LH05 was unable to combine with an anti-peptide antibody (LHP03) directed to residues 43-52 of hLHbeta. Thus, LH05 recognizes an epitope partly overlapping with those recognized by HT13 and LHP03. Using various hybrid molecules, we showed that the human alpha-subunit plays a critical role in the assembly of the epitope that, in contrast, contains amino acid residues conserved in the various beta-subunit of several species. Together, our results suggest that the amino acids Leu49-Pro50, Tyr59-Arg60 and Leu86-Ser87 in the hLHbeta and the alpha64-76 region are probably included in the epitope recognized by LH05 which appeared to be not accessible on the CG/LH receptor.

PMID: 9397932 [PubMed - indexed for MEDLINE]

☐ 1: J Reprod Immunol. 1996 May;30(2-3):133-49. Related Articles, Links

Detection of epitopes on follicle-stimulating hormone and FSH-antiserum-induced suppression of bioactivity of follicle-stimulating hormone and luteinizing hormone.

Westhoff WE, Slootstra JW, Puijk WC, Kuperus D, Flinterman JF, Schaaper WM, Oonk HB, Meloen RH.

Institute for Animal Science and Health, Department of Molecular Recognition, AB Lelystad, The Netherlands.

There are currently two major approaches to hormonal male contraception. One relies on testosterone (analogs) either alone or in combination with gonadotropin releasing hormone (GnRH) (analogs or immunizations), the other on immunizations against follicle-stimulating hormone (FSH). Theoretically, the latter method will suppress spermatogenesis whilst not interfering with libido. An absolute requirement is, however, that an anti-FSH vaccine does not include anti-luteinizing hormone (LH) antibodies (LH being responsible for the induction of testosterone which is necessary to maintain libido). In this report we show that when whole FSH is used for vaccination, in most cases in addition to biological activity against FSH, anti-LH activity is also induced. By systematic analysis of the antisera raised with FSH using systematic epitope scanning (PEPSCAN) we found differences between the FSH-specific and FSH-nonspecific sera. Only the FSH-specific antiserum contained antibodies that recognized amino acid sequence 37-55 on the beta-subunit in a linear manner. Because antibodies against this epitope have not been found in the cross-reactive sera this epitope forms a prime candidate for an anti-FSH contraceptive vaccine.

PMID: 8816329 [PubMed - indexed for MEDLINE]

J Reprod Immunol. 1996 May;30(2-3):133-49. Related Articles, Links

Detection of epitopes on follicle-stimulating hormone and FSH-antiserum-induced suppression of bioactivity of follicle-stimulating hormone and luteinizing hormone.

Westhoff WE, Slootstra JW, Puijk WC, Kuperus D, Flinterman JF, Schaaper WM, Oonk HB, Meloen RH.

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PMID: 8816329 [PubMed - indexed for MEDLINE]

Immunometrics

Products

- Diagnostic Kits
  - Luteinising Hormone
  - **Follicle Stimulating Hormone**
  - Prolactin
  - Human Chorionic Gonadotrophin
  - Alpha Feto-Protein
  - Thyroid Stimulating Hormone
  - Progesterone
  - Testosterone
  - Estradiol
  - Triiodothyronine
  - Thyroxine
  - Estrone-3-Glucuronide
  - Pregnanediol-3-alpha-Glucuronide
  - Levonorgestrel (D-Norgestrel)
  - Norethisterone (Norethindrone)
  - Medroxyprogesterone Acetate
  - Ethinyl Estradiol
- Primary reagents
- Magnetic separator
- Kit Product Codes

FSH EIA kit

- **high quality**
  - developed for the World Health Organization (WHO)
  - used in WHO international research projects
  - used in 25 countries worldwide
  - performs well in national and international EQA Schemes
- **high performance**
  - detection limit <0.2 IU/L WHO IRP 78/549
  - between-assay CV better than 10% in range 1 - 200 IU/L
- **low cost**
  - all reagents are provided - only test tubes and water are required
  - uses inexpensive, robust equipment
  - each kit is sufficient for 100 assay tubes
- **convenient**
  - magnetic separation
  - 2 hours total incubation time
  - shelf life of 1 year at 4-8°C

For information and prices please contact us.

Principle

The assay is an immunometric ('sandwich') EIA for the quantitative measurement of FSH in human serum or plasma. FSH in the sample is bound by 2 monoclonal anti-FSH antibodies directed at different epitopes. One antibody is attached to magnetic particles and the other is labelled with alkaline phosphatase. The assay has four main stages:

- Magnetic anti-FSH is incubated with sample (100 µL) for 15 minutes at 37°C followed by a magnetic wash step.
- The magnetic particles are incubated with alkaline phophatase labelled anti-FSH for 1 hour at 37°C followed by 2 magnetic wash steps.
- A coloured enzyme substrate is incubated with the particles for 30 minutes at 37°C. The presence of alkaline phosphatase causes a colour change from yellow to pink. The reaction is terminated by addition of Stop Buffer.
- The tubes are then placed in the spectrophotometer or colorimeter. The optical density (at 550nm or 492nm) of each tube can be measured and the results calculated using a data processing program.

See diagram popup.

Kit Parameters

Incubation times	15 mins + 1 hr + 30 mins
Standard Range	0-200 IU/LStandardised against WHO IRP 78/549
Normal Ranges	

Female day 3-5	3.3±12.9 IU/L
Female peak FSH	11.3±30.1 IU/L

Male	2.3±11.3 IU/L

Detection Limit 0.2 IU/L

Assay Reproducibility

Within-batch ±1SD	
IU/L	%CV
3.8±0.10	2.5
12.3±0.5	3.7
17.7±0.3	1.9
30.0±0.6	2.1

Assay Reproducibility

Between-batch ±1SD	
IU/L	%CV
4.1±0.2	5.2
12.4±1.0	8.3
17.1±0.8	4.5
32±1.1	3.5

Kit Contains

FSH EIA Standards - see (*)
FSH EIA Magnetic Antibody
FSH EIA Enzyme Labelled Antibody
FSH EIA Assay Buffer
FSH EIA Wash Buffer
EIA Substrate Reagent
EIA Substrate Buffer
EIA Stop Buffer
EIA Internal QC Sample
Instruction booklet

(*) calibrated against WHO IRP 78/549.

Equipment Required

Test tubes, pipettes, multivortex mixer, magnetic separator, 37°C water-bath and a colorimeter or spectrophometer.

Pept Res. 1996 Jul-Aug;9(4):195-202. Related Articles, Links

Studies on the delineation of the hormone-specific antigenic determinants of human follicle-stimulating hormone.

Lal D, Mahale SD, Iyer KS.

Institute for Research in Reproduction (I.C.M.R.), Parel, Bombay, India.

Human follicle-stimulating hormone (hFSH) is a key hormone regulating both male and female reproduction. The present study attempts to delineate the hFSH-specific antigenic determinants on its beta-subunit. Predictive methods were used to identify the potential surface-oriented regions of hFSH-beta. Peptides corresponding to these regions, namely, 31-52, 66-75 and 86-95 hFSH-beta, were synthesized and conjugated to diphtheria toxoid. Antipeptide antibodies, elicited in rabbits by immunization with the conjugates, were screened for their ability to bind to hFSH-beta and hFSH. Anti-31-52 hFSH-beta antisera bound to both hFSH-beta and hFSH, whereas anti-66-75 and anti-86-95 hFSH-beta antisera did not show any detectable binding. Furthermore, screening of anti-hFSH antisera showed significant binding only to 31-52 hFSH-beta. These results identify the region 31-52 hFSH-beta as a hormone-specific antigenic determinant of hFSH.

PMID: 8914167 [PubMed - indexed for MEDLINE]

All: 1 Review: 1

1: Trends Biotechnol. 1992 Dec;10(12):427-32. Related Articles, Links

Assaying glycoprotein hormones--the influence of glycosylation on immunoreactivity.

Storring PL.

Division of Endocrinology, National Institute for Biological Standards and Control, Potters Bar, Herts., UK.

The epitopes of the human glycoprotein hormones (follicle-stimulating hormone [hFSH], luteinizing hormone [hLH], chorionic gonadotrophin [hCG], thyroid-stimulating hormone [hTSH] and erythropoietin [hEPO]) appear to consist only of peptide components. Their interactions with antibodies, however, are influenced by their bulky and often highly charged carbohydrate moieties. Thus, isoforms of these hormones (the majority of which are glycoforms) differ in their specific immunoreactivities as well as in their specific in vivo and in vitro bioactivities. This can create difficulties for the standardization of immunoassays as the isoform composition of a hormone depends both on its source and method of isolation.

Publication Types:
- Review

PMID: 1283302 [PubMed - indexed for MEDLINE]

Am J Reprod Immunol. 1993 Jan;29(1):48-55. Related Articles, Links

Immunoneutralization of heterodimeric FSH using FSH beta subunit as the immunogen.

Butterstein GM, Sachar D, Dias JA.

Department of Biology, Union College, Schenectady, New York.

PROBLEM: Immunization with beta subunit of gonadotropin may elicit antibody formation to endogenous heterodimeric gonadotropin and result in reproductive unresponsiveness. The objectives of this study were to determine if antibodies produced in rats following immunization with human follicle stimulating hormone beta-subunit (hFSH-beta) could bind to and immunoneutralize heterodimeric FSH, and to elucidate the immunoneutralizing epitope. METHOD: Mature female Sprague-Dawley rats received subcutaneous injections of 10 micrograms of hFSH-beta emulsified in complete Freund's adjuvant, while control animals received only adjuvant. Animals received 10 micrograms hFSH-beta booster injections emulsified in incomplete Freund's adjuvant 2, 4, 11, and 21.5 wk after the initial immunization. RESULTS: Immunization with hFSH-beta produced appreciable antibody titers against human FSH (hFSH) as measured in enzyme-linked immunosorbent assays (ELISA) of immunized rat sera. A more modest titer to rat (rFSH) and no antibody response to rat Luteinizing Hormone (rLH) was observed, thus confirming the specificity of the immune response. Titers against hFSH increased throughout the study. Rat anti-hFSH-beta sera was tested to determine its ability to inhibit binding (immunoneutralizing) of 125I-hFSH to the FSH receptor. Continued immunization resulted in all animals producing immunoneutralizing antibodies. Immunization of rats also resulted in disrupted estrous cycles, but only animals with subsequent titers high enough to completely block binding of FSH to its receptor failed to conceive. In order to assess the immunoneutralizing epitope, antisera were tested in a peptide ELISA. Peptides used in the ELISA corresponded to amino acids that spanned the entire hFSH beta sequence. It was found that antibodies from all rats immunized with hFSH beta bound to amino acids within hFSH-beta 33-53. CONCLUSIONS: Collectively, these data suggest that amino acids within hFSH-beta 33-53 are necessary but not sufficient to confer immunocontraception. Amino acids within this linear sequence appear in a variety of epitopes of hFSH-beta and hFSH, only some of which are immunoneutralizing. (Am J Reprod Immunol. 1993; 29:000-000.)

MATERIAL SAFETY DATA SHEET FOR IMMULITE FOLLICLE STIMULATING HORMONE (FSH) LKFS1,5

1 PRODUCT IDENTIFICATION

TRADE NAME: IMMULITE Follicle Stimulating Hormone (FSH) In-vitro Diagnostic Test Kit **PRODUCT CODE: LKFS1,5**

MANUFACTURER/SUPPLIER: EURO/DPC Limited, Glyn Rhonwy, Llanberis, Caernarfon, Gwynedd LL55 4EL, United Kingdom
Phone Number: 44-1-286-871-871 Fax Number: 44-1-286-871-802
Phone Number in U.S.: (800) 372-1782 Emergency Phone Number in U.S.: (800) 372-1782

PRODUCT COMPONENTS & CATALOG NUMBERS: FSH Test Units: LFS1; Reagent LFS2; Adjustors LFSL & LFSH

PRODUCT FORMAL NAME: Diagnostic Reagent **PRODUCT CHEMICAL NAME:** Aqueous Solution

2 HAZARDOUS INGREDIENTS

Kit Component(s): LFS2, LFSL & LFSH

Hazardous Component	Percent	CAS Number	EEC Classification	Symbol	Index #
Sodium Azide (NaN_3)	<0.1% w/w	26628-22-8	–	–	–

3 HAZARD IDENTIFICATION

Sodium Azide is a toxic substance. Avoid contact with components, which contain sodium azide, and do not ingest. An accumulation of sodium azide may result in a reaction with lead or copper plumbing to form an explosive metal azide complex. If drain disposed, dilute and flush with a copious amount of running water to prevent azide build-up. Dangerous when in contact with acid.

4 FIRST AID MEASURES

EYE CONTACT: Flush with copious amounts of fresh water for at least 15 minutes.

SKIN CONTACT: Wash well with mild soap and copious amounts of fresh water. Remove any contaminated clothing. Flush skin surface with additional water.

INGESTION: Flush mouth with copious amounts of water. Do not swallow rinse water.

INHALATION: Remove victim to fresh air. If breathing is labored, administer oxygen as needed. If victim is not breathing, administer artificial respiration or CPR.

If warranted, seek medical attention. If possible, save sample of material that caused reaction for use in determination of appropriate treatment.

5 FIRE EXTINGUISHING MEASURES

Use extinguishing media appropriate to surrounding fire. No special equipment or procedures are required.

6 ACCIDENTAL RELEASE MEASURES

Absorb spills of reagents and patient samples with absorbent paper, taking care not to spread the material. Clean spill area with a freshly made 0.5% sodium hypochlorite (bleach) solution. Discard all materials used to absorb spill and disinfect area into biohazard waste collection for proper disposal.

7 HANDLING AND STORAGE

HANDLING: Do not eat, drink, smoke or apply cosmetics in laboratory areas. Do not pipet patient samples or reagents by mouth. Avoid splashing or aerosol formation. Use all reagents in accordance with the relevant package insert. Avoid high temperatures and keep from freezing during transport.

STORAGE: Store all reagents as directed in the relevant package insert.

MATERIAL SAFETY DATA SHEET FOR IMMULITE FOLLICLE STIMULATING HORMONE (FSH) LKFS1,5

8 EXPOSURE CONTROL/PERSONAL PROTECTION

Wear appropriate personal protective equipment when working with reagents or patient specimens, including lab coats, disposable gloves and eye protection. Avoid hand/mouth contact. Wash hands as soon as possible after handling reagents or patient specimens.

Control Parameters of Hazardous Ingredients:

Sodium Azide: CAS # 26628-22-8, RTECS # VY805000, TLV-Ceiling=0.3mg/m, NIOSH (the concentration of sodium azide in this product is well below the TLV shown above). Threshold limit value 1.0 ppm, TDLo (oral) 710mcg/kg, female 3mg/kg, LDLo (oral) 29mg male, LDLo (oral) 786 mg female.

9 PHYSICAL & CHEMICAL PROPERTIES

Physical State: Liquid	Color: Clear	Odor: None	pH: N/A
Boiling Point: 100°C	Melting Point: 0°C	Flash Point: N/A	Inflammability: N/A
Autoinflammability: N/A	Explosiveness: N/A	Oxidizing Properties: N/A	
Vapor Pressure: N/A	Relative Density: N/A	Solubility in water: Complete	

10 STABILITY & REACTIVITY

The reagents in the kit are stable under the storage conditions described in the package insert. Hazardous decomposition will not occur. There are no known strong incompatibilities.

11 TOXICOLOGICAL INFORMATION

Not applicable

12 ECOLOGICAL INFORMATION

Not applicable

13 DISPOSAL

Dispose in accordance with applicable laws. If drain disposed, dilute and flush with a copious amount of running water to prevent azide build-up (See Section 3).

14 TRANSPORT INFORMATION

Proper Shipping Name: In vitro diagnostic reagents
Hazard Class: None
Identification Number: None

15 REGULATORY INFORMATION

Pursuant to U.S. OSHA regulations and the EEC Directive Number 88/379, the only hazardous ingredients associated with this product are those listed in Section 2 above.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The user should determine the suitability of this information for the intended use of the product and adopt appropriate safety precautions. DPC shall not be held liable for any damage resulting from handling or from contact with the above product. Contact DPC for further information.

Date MSDS first initiated: January 14th 1993
Date MSDS Revised: March 9, 2000

Prepared By: B. Gonzalez

Sections Revised: 1, 3, 5, 14, 15

IMMULITE®

FSH

**For use on the IMMULITE®
and IMMULITE® 1000 systems**

DPC

IMMULITE®/IMMULITE® 1000 FSH

English

Intended Use: For *in vitro* diagnostic use with the IMMULITE and IMMULITE 1000 Analyzers — for the quantitative measurement of follicle stimulating hormone (FSH, follitropin) in serum, as an aid in the diagnosis and treatment of pituitary and gonadal disorders.

Catalog Number: **LKFS1** (100 tests), **LKFS5** (500 tests)

Test Code: **FSH** Color: **Light Gray**

CDC Analyte Identifier Code: 1908
CDC Test System Identifier Code: 10159
CLIA Complexity Category: Moderate

Summary and Explanation

Follicle stimulating hormone (follitropin, FSH) is secreted by the β-cells of the anterior pituitary under the control of the gonadotropin releasing hormone produced in the hypothalamus. FSH facilitates the development and maintenance of gonadal tissues, which synthesize and secrete steroid hormones. Circulating levels of FSH are controlled by a negative feedback mechanism on the hypothalamus by steroidal hormones. Although FSH and LH are required for normal sexual function in both males and females, the secretory patterns are very different for the two sexes.

In mature females, FSH initiates the growth and development of ovarian follicles. During ovulation, when the follicle is ruptured, the follicle, now called the corpus luteum, secretes estradiol and progesterone, which control the circulating levels of FSH by a negative feedback effect on the hypothalamus. In menopause, with diminished ovarian function, there is a resulting decrease in estradiol secretion. Due to the lack of a negative feedback effect, with diminished estradiol, the circulating FSH levels become significantly increased.

In the mature male, FSH is associated with the stimulation and maintenance of spermatogenesis. Testosterone and estradiol have the role of providing the negative feedback signal to the hypothalamus for controlling the release of FSH. Infertility in males may be due to hypogonadism as a result of primary testicular failure. Testicular failure may be functional failure to mature, or a result of germ cell damage. Whatever the etiology, the conditions of hypogonadism have the net result of dramatically raising the circulating FSH levels, due to the lack of a negative feedback effect.

Principle of the Procedure

IMMULITE/IMMULITE 1000 FSH is a solid-phase, two-site chemiluminescent immunometric assay.

Incubation Cycles: 1 × 30 minutes.

Specimen Collection

The use of an ultracentrifuge is recommended to clear lipemic samples.

Hemolyzed samples may indicate mistreatment of a specimen before receipt by the laboratory; hence the results should be interpreted with caution.

EDTA plasma should not be used as a sample type.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Blood collection tubes from different manufacturers may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators and/or anticoagulants. IMMULITE/IMMULITE 1000 FSH has not been tested with all possible variations of tube types. Consult the section on Alternate Sample Types for details on tubes that have been tested.

Volume Required: 50 µL serum. (Sample cup must contain at least 100 µL more than the total volume required.)

Storage: 7 days at 2–8°C or
2 months at –20°C.

Warnings and Precautions

For *in vitro* diagnostic use.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. The barcode labels are needed for the assay.

FSH Test Units (LFS1)
Each barcode-labeled unit contains one bead coated with monoclonal murine anti-FSH. Stable at 2–8°C until expiration date.
LKFS1: 100 units. **LKFS5:** 500 units.

Allow the Test Unit bags to come to room temperature before opening. Open by cutting along the top edge, leaving the ziplock ridge intact. Reseal the bags to protect from moisture.

FSH Reagent Wedge (LFS2)
With barcode. 7.5 mL alkaline phosphatase (bovine calf intestine) conjugated to murine monoclonal anti-FSH antibody in buffer, with preservative. Store capped and refrigerated: stable at 2–8°C until expiration date. Recommended usage is within 30 days after opening when stored as indicated.
LKFS1: 1 wedge. **LKFS5:** 5 wedges.

FSH Adjustors (LFSL, LFSH)
Two vials (Low and High), 3.0 mL each, of FSH in a nonhuman serum matrix, with preservative. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.
LKFS1: 1 set. **LKFS5:** 2 sets.

Kit Components Supplied Separately

FSH Sample Diluent (LFSZ)
For the manual dilution of patient samples. One vial containing 25 mL of an FSH-free nonhuman serum matrix, with preservative. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.

LSUBX: Chemiluminescent Substrate
LPWS2: Probe Wash Module
LKPM: Probe Cleaning Kit
LCHx-y: Sample Cup Holders (barcoded)
LSCP: Sample Cups (disposable)
LSCC: Sample Cup Caps (optional)

CON6: Tri-level, multi-constituent control

Also Required
Sample transfer pipets, distilled or deionized water, controls.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE or IMMULITE 1000 Operator's Manual.

See the IMMULITE or IMMULITE 1000 Operator's Manual for: preparation, setup, adjustment, assay and quality control procedures.

Visually inspect each Test Unit for the presence of a bead before loading it onto the system.

Recommended Adjustment Interval: 4 weeks.

Quality Control Samples: Use controls or sample pools with at least two levels (low and high) of FSH.

Expected Values

Reference ranges were generated using IMMULITE FSH (mono/poly) in a multi-national study involving women in apparent good health (age: 16 – 44 years), who volunteered to have blood samples drawn, on a daily basis, throughout one complete ovulatory cycle. (See "Menstrual Cycle" graph.)

Ovulatory Cycles	n*	FSH, mIU/mL Median	Central 95%
Follicular Phase	54 (762)	6.2	2.8 – 11.3
Follicular Phase, Days 2 to 3	54 (108)	6.6	3.0 – 14.4
Midcycle	54 (54)	13.6	5.8 – 21
Luteal Phase	54 (604)	3.4	1.2 – 9.0

*Number of subjects (total number of results)

The table below summarizes results for adult males and (untreated) postmenopausal females from studies with IMMULITE FSH (mono/poly). The ranges for women on oral contraceptives and for postmenopausal women on ERT are based on the assay's relationship to DPC's Coat-A-Count FSH IRMA.

Group	n	FSH, mIU/mL Median	Central 95%
Adult Males	135	3.8	0.7 – 11.1
Adult Females:			
Postmenopausal*	76	90.5	21.7 – 153
Postmenopausal (ERT)	16	27	9.7 – 111
Oral Contraceptives	12	1.7	ND – 4.9

*Preliminary
ND: not detectable

A cross-sectional study of pediatric values performed with IMMULITE FSH (mono/poly) at a "wellness" clinic in the southwestern United States yielded the following results.

Group	Age (yr)	n	FSH, mIU/mL Median	Central 95%
Females	Cord	30	ND	
	0.1 – 3	57	2.3	0.11 – 13
	4 – 9	28	0.8	0.11 – 1.6
Males	Cord	37	0.24	ND – 1.2
	0.1 – 3	72	0.6	ND – 5.5
	4 – 9	31	0.23	ND – 1.9
Combined	Cord	67	0.11	ND – 1.1
	0.1 – 3	129	1.1	ND – 10
	4 – 9	59	0.5	ND – 1.8

ND: not detectable

Consider these limits as *guidelines* only. Each laboratory should establish its own reference ranges.

Limitations

In certain cases of infertility, treatment with human gonadotropins poses a potential problem for the accurate measurement of FSH levels. The FSH that is administered can cause the patient to produce antibodies to FSH which will interfere directly with the assay.

Because of pulsatile secretion, samples obtained within the same day from the same patient may fluctuate widely within the reference range, reflecting physiological variation rather than errors in technique or methodology.

Because EDTA would have a significant effect on results, it should not be used as an anticoagulant.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Performance Data

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed in mIU/mL. (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Calibration Range: up to 170 mIU/mL (8.9 ng/mL) [WHO 2nd IRP 78/549] [interim replacement code 94/632].

Analytical Sensitivity: 0.1 mIU/mL

High-dose Hook Effect: None up to 50,000 mIU/mL.

Precision: Samples were assayed in duplicate in 40 runs for a total of 80 replicates. (See "Precision" table.)

Linearity: Samples were assayed under various dilutions. (See "Linearity" table for representative data.)

Recovery: Samples spiked 1 to 19 with three FSH solutions (150, 600 and 1,800 mIU/mL) were assayed. (See "Recovery" table for representative data.)

Specificity: The antibody is highly specific for FSH. (See "Specificity" table.)

Bilirubin: Presence of bilirubin in concentrations up to 200 mg/L has no effect on results, within the precision of the assay.

Hemolysis: Presence of hemoglobin in concentrations up to 600 mg/dL has no effect on results, within the precision of the assay.

Lipemia: Presence of triglycerides in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay.

Alternate Sample Type: To assess the effect of alternate sample types, blood was collected from 24 volunteers into plastic Becton Dickinson plain serum, heparinized, EDTA and SST® vacutainer tubes. All samples were assayed by the IMMULITE FSH procedure

(Heparin) = 1.10 (Serum) + 0.65 mIU/mL

(SST) = 0.94 (Plain Tubes) + 0.12 mIU/mL

(EDTA) = 0.88 (Serum) + 0.57 mIU/mL

Means:
11.7 mIU/mL (Serum)
13.5 mIU/mL (Heparin)
11.5 mIU/mL (SST)
10.8 mIU/mL (EDTA)

EDTA plasma should not be used as a sample type.

Method Comparison: The IMMULITE FSH mono/mono assay was compared to DPC's IMMULITE FSH mono/poly assay on 431 samples. (Concentration range: approximately 0.7 to 130 mIU/mL. See graph.) By linear regression:

(IML m/m) = 0.90 (IML m/p) + 0.35 mIU/mL
r = 0.990

Means:
31.4 mIU/mL (IMMULITE mono/mono)
34.5 mIU/mL (IMMULITE mono/poly)

References

1) Santner S, Santen R, Kulin H, Demers L. A model for validation of radioimmunoassay kit reagents: measurement of follitropin and lutropin in blood and urine. Clin Chem 1981;27:1892-5. 2) Odell W, et al. Radioimmunoassay for luteinizing hormone in human plasma or serum. J Clin Invest 1967;46:248-55. 3) Davidsohn I, Henry J, editors. Clinical diagnosis by laboratory methods. 15th ed. Philadelphia: W.B. Saunders, 1974: 704. 4) Nankin H, et al. Repetitive luteinizing hormone elevations in serum of normal men. J Clin Endo Metab 1972;33:558-60. 5) Beitens I, et al. Gonadotropin determinations in timed 3-hour urine collections during the menstrual cycle and LHRH testing. J Clin Endo Metab 1976;43:46-55. 6) Chipman J, et al. Interrelationship of plasma and urinary gonadotropins: correlations for 24 hours, for sleep/wake periods, and for 3 hours after luteinizing hormone-releasing hormone stimulation. Clin Endo Metab 1981;52:225-30. 7) Kulin H, et al. Integration of pulsatile gonadotropin secretion by timed urinary measurements: an accurate and sensitive 3-hour test. J Clin Endo Metab 1975;40:783-9. 8) Kulin H, Santner S. Timed urinary gonadotropin measurements in normal infants, children, and adults, and in patients with disorders of sexual maturation. J Pediatrics 1977;90:760-5. 9) Urban M, et al. Comparison of estimates of gonadotropin levels by isolated blood samples, integrated blood concentrations, and timed urinary fractions. J Clin Endo Metab 1979;48:732-5. 10) Rebar R, Yen S. In: Dorothy Krieger, editor. Endocrine rhythms. New York: Raven Press, 1979. 11) Odell WD, et al. Radioimmunoassay for human follicle-stimulating hormone: physiological studies. J Clin Invest 1968;47:2551-62. 12) Sutaria UE, Crooke AC. Selection of patients for treatment with human gonadotrophins. Int J Fertil 1971;16:42-6. 13) Babson, AL. The IMMULITE Automated Immunoassay System. J Clin Immunoassay 1991;14:83-88. 14) National Committee for Clinical Laboratory Standards. Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard. 4th ed. NCCLS Document H3-A4, Wayne, PA: NCCLS, 1998.

Technical Assistance

In the United States, contact DPC's Technical Services department.
Tel: 800.372.1782 or 973.927.2828
Fax: 973.927.4101. Outside the United States, contact your National Distributor.

Manufactured by EURO/DPC Ltd. under a Quality System registered to ISO 13485:2003.

Tables and Graphs

Precision (mIU/mL)

		Within-Run[1]		Total[2]	
	Mean[3]	SD[4]	CV[5]	SD	CV
1	5.7	0.15	2.6%	0.33	5.8%
2	19.4	0.59	3.0%	1.04	5.4%
3	38.5	0.89	2.3%	2.13	5.5%
4	58.8	2.14	3.6%	3.60	6.1%
5	87.0	3.18	3.7%	5.81	6.7%

Linearity (mIU/mL)

	Dilution[1]	Observed[2]	Expected[3]	%O/E[4]
1	8 in 8[5]	8.95	—	—
	4 in 8	4.50	4.48	100%
	2 in 8	2.36	2.24	105%
	1 in 8	1.16	1.12	104%
2	8 in 8	13.6	—	—
	4 in 8	7.42	6.80	109%
	2 in 8	3.73	3.40	110%
	1 in 8	1.95	1.70	115%
3	8 in 8	27.8	—	—
	4 in 8	14.9	13.9	107%
	2 in 8	7.67	6.95	110%
	1 in 8	3.81	3.48	109%
4	8 in 8	53.8	—	—
	4 in 8	26.8	26.9	100%
	2 in 8	13.9	13.5	103%
	1 in 8	7.11	6.73	106%
5	8 in 8	68.1	—	—
	4 in 8	37.2	34.1	109%
	2 in 8	21.0	17.0	124%
	1 in 8	11.1	8.51	130%
6	8 in 8	109	—	—
	4 in 8	53.1	54.5	97%
	2 in 8	28.7	27.3	105%
	1 in 8	14.7	13.6	108%

Recovery (mIU/mL)

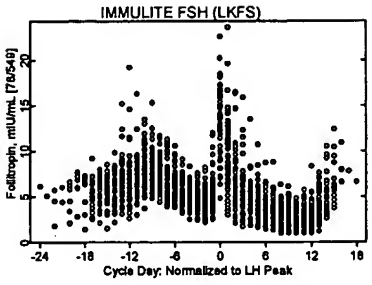
	Solution[1]	Observed[2]	Expected[3]	%O/E[4]
1	—	1.45	—	—
	A	9.42	8.88	106%
	B	30.8	31.4	98%
	C	98.1	91.4	107%
2	—	4.34	—	—
	A	12.9	11.6	111%
	B	34.6	34.1	101%
	C	107	94.1	114%
3	—	4.88	—	—
	A	12.5	12.1	103%
	B	33.4	34.6	97%
	C	105	94.6	111%
4	—	52.8	—	—
	A	60.0	57.7	104%
	B	83.8	80.2	104%
	C	169	140	121%

Specificity

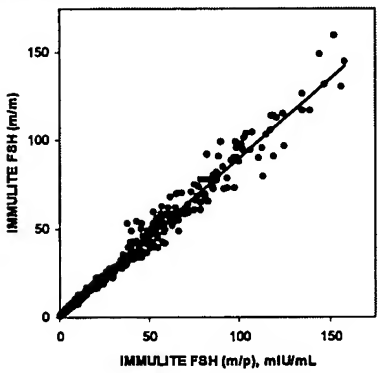
	Added[2]		Apparent FSH	
Compound[1]		ng/mL	ng/mL	% Cross reactivity[3]
HCG	2,000	215	ND	ND
mIU/mL	200,000	21,538	ND	ND
TSH	10	2.0	ND	ND
uIU/mL	200	40.5	ND	ND
LH	75	7.6	ND	ND
mIU/mL	300	30.5	ND	ND
Prolactin	25	25	ND	ND
ng/mL	200	200	ND	ND
HGH	5.0	5.0	ND	ND
ng/mL	50	50	ND	ND
HPL	10,000	10,000	ND	ND
ng/mL	50,000	50,000	ND	ND

ND: not detectable.[4]

Menstrual Cycle



Method Comparison



(IML m/m) = 0.90 (IML m/p) + 0.35 mIU/mL
r = 0.990

Deutsch. **Intraassay Precision**: [1]Mittelwert, [2]SD (Standardbereich), [3]CV (Variationskoeffizient). **Interassay Precision**: [1]Mittelwert, [2]SD (Standardbereich), [3]CV (Variationskoeffizient). **Linearity**: [1]Verdünnung, [2]Beobachtet (B), [3]Erwartet (E), [4]% B/E, [5]8 in 8. **Recovery**: [1]Lösung, [2]Beobachtet (B), [3]Erwartet (E), [4]% B/E. **Specificity**: [1]Verbindung, [2]zugesetzte Menge, [3]Ausgewiesene Konzentration, [4]% Kreuzreaktivität, [5]NN: Nicht nachweisbar. **Method Comparison**: FSH: FSH.

Español. **Intraassay Precision**: [1]Media, [2]DS, [3]CV. **Interassay Precision**: [1]Media, [2]DS, [3]CV. **Linearity**: [1]Dilución, [2]Observado (O), [3]Esperado (E), [4]%O/E, [5]8 en 8. **Recovery**: [1]Solución, [2]Observado (O), [3]Esperado (E), [4]%O/E. **Specificity**: [1]Compuesto, [2]Cantidad añadida, [3]Concentración aparente, [4]% Reacción cruzada, [5]ND: no detectable. **Method Comparison**: FSH: FSH.

Français. **Intrraassay Precision**: [1]Moyenne, [2]SD, [3]CV. **Interassay Precision**: [1]Moyenne, [2]SD, [3]CV. **Linearity**: [1]Dilution, [2]Observé (O), [3]Attendu (A), [4]%O/A, [5]8 dans 8. **Recovery**: [1]Solution, [2]Observé (O), [3]Attendu (A), [4]%O/A. **Specificity**: [1]Composé, [2]ajouté, [3] Concentration apparente, [4]Réaction croisée%. [5]ND: non détectable. **Method Comparison**: FSH: FSH.

Italiano. **Intraassay Precision**: [1]Media, [2]SD (Deviazione Standard), [3]CV (Coefficiente di Variazione). **Interassay Precision**: [1]Media, [2]SD (Deviazione Standard), [3]CV (Coefficiente di Variazione). **Linearity**: [1]Diluizione, [2]Osservato (O), [3]Atteso (A), [4]%O/A, [5]8 in 8. **Recovery**: [1]Soluzione, [2]Osservato (O), [3]Atteso (A), [4]%O/A. **Specificity**: [1]Composto, [2]quantità aggiunta, [3] Concentrazione apparente, [4]Percentuale di Crossreattività, [5]ND: non determinabile. **Method Comparison**: FSH: FSH.

Português. **Intraassay Precision**: [1]Média, [2]Desvio padrão, [3]Coeficiente de variação. **Interassay Precision**: [1]Média, [2]Desvio padrão, [3]Coeficiente de variação. **Linearity**: [1]Diluição, [2]Observado (O), [3]Esperado (E), [4]%O/E, [5]8 em 8. **Recovery**: [1]Solução, [2]Observado (O), [3]Esperado (E), [4]%O/E. **Specificity**: [1]Composto, [2]Quantidade adicionada, [3]Apparent Concentration, [4]Percentagem de reacção cruzada, [5]ND: não detectável. **Method Comparison**: FSH: FSH.

Deutsch

FSH IMMULITE

Anwendung: Zur *in vitro*-Diagnostik unter Verwendung der IMMULITE und IMMULITE 1000 Systeme – zur quantitativen Messung von Follikel stimulierendes Hormon (Follitropin, FSH) im Serum, als Hilfe bei der Diagnose und Behandlung von Störungen der Hypophysen- und Gonadenfunktion.

Artikelnummern:
LKFS1 (100 Tests)
LKFS5 (500 Tests)
Testcode: **FSH** Farbe: **hellgrau**

Klinische Relevanz

Follikel stimulierendes Hormon (Follitropin, FSH) wird durch die B-Zellen des vorderen Hypophyselappens ausgeschieden und unterliegt der Kontrolle durch das Gonadotropin Releasing Hormon des Hypothalamus. FSH ermöglicht die Entwicklung und Erhaltung des gonadalen Gewebes, das die Steroidhormone synthetisiert und freisetzt. Die zirkulierenden FSH-Spiegel werden durch die steroidalen Hormone über einen negativen Rückkopplungsmechanismus auf den

Hypothalamus gesteuert. Obwohl FSH und LH zur normalen sexuellen Funktion beider Geschlechter erforderlich sind, zeigen sich erhebliche Unterschiede im sekretorischen Muster für Männer und Frauen.

Bei Frauen im fertilen Alter, initiiert das FSH das Wachstum und die Entwicklung ovarieller Follikel. Während der Ovulation, nach dem Aufbrechen des Follikels, produziert der Follikel, jetzt Corpus luteum, Östradiol und Progesteron. Diese beiden Hormone regulieren die zirkulierenden FSH-Spiegel über eine negative Rückkopplung auf den Hypothalamus. In der Menopause kommt es auf Grund der verringerten ovariellen Funktion zu einer merklichen Abschwächung der Östradiol-Freisetzung. Das Fehlen einer Östradiol gesteuerten negativen Rückkopplung, verursacht einen beträchtlichen Anstieg der FSH-Konzentrationen.

Im erwachsenen Mann ist das FSH assoziiert mit der Stimulation und Aufrechterhaltung der Spermatogenese. Testosteron und Östradiol haben die Aufgabe das negative Rückkopplungssignal zum Hypothalamus zu unterstützen, um die Freisetzung des FSH zu kontrollieren. Die Infertilität bei Männern kann in einem Hypogonadismus begründet sein, der auf primäre testikuläre Störungen zurückzuführen ist. Testikuläre Störungen können funktionelle Störungen in der Reifung zugrundeliegen. Aber auch die Zerstörung von Keimzellen kann zu gleichen Symptomen führen. Was auch immer die ätiologische Ursache ist, Hypogonadismus führt im Ergebnis zu einem dramatischen Anstieg der meßbaren FSH-Konzentrationen, da der negative Rückkopplungseffekt nicht greifen kann.

Methodik

Der IMMULITE/IMMULITE 1000 FSH ist ein Festphasen-, Zweischritt-, Chemilumineszenz-, Immunometrischer Assay.

Inkubationszyklen: 1 × 30 min.

Probengewinnung

Der Einsatz einer Ultrazentrifuge wird zur Klärung von lipämischen Proben empfohlen.

Bei hämolysierten Proben besteht die Möglichkeit einer unsachgemäßen Handhabung vor Eintreffen im Labor, daher sind die Ergebnisse zurückhaltend zu interpretieren.

EDTA-Plasma ist als Probenart nicht geeignet.

Die Zentrifugation der Serumproben vor dem völligen Abschluss der Gerinnung kann zu Fibringerinnseln führen. Um fehlerhaften Analysenergebnissen infolge von Gerinnseln vorzubeugen, ist sicherzustellen, dass die Gerinnung vor der Zentrifugation der Proben vollständig abgeschlossen ist. Insbesondere Proben von Patienten unter Antikoagulantien-therapie können eine verlängerte Gerinnungszeit aufweisen.

Blutentnahmeröhrchen von verschiedenen Herstellern können differierende Werte verursachen. Dies hängt von den verwendeten Materialien und Additiven (Gel oder physische Trennbarrieren, Gerinnungsaktivatoren und /oder Antikoagulantien) ab. IMMULITE/IMMULITE 1000 FSH sind nicht mit allen möglichen Röhrchenvariationen ausgetestet worden. Details der getesteten Röhrchenarten sind dem Kapitel "Alternative Probenarten" zu entnehmen.

Erforderliche Menge: 50 µl Serum. (Inhalt der Probenschale muss mindestens 100 µl über der erforderlichen Gesamtmenge liegen.)

Lagerung: 7 Tage bei 2–8°C oder 2 Monate bei –20°C.

Hinweise und Vorsichtsmaßnahmen

Zur *In-vitro*-Diagnostik.

Reagenzien: Bei 2–8°C lagern. Unter Einhaltung der geltenden gesetzlichen Vorschriften entsorgen.

Die generell geltenden Vorsichtsmaßnahmen sind einzuhalten und alle Komponenten als potenziell infektiös zu behandeln. Alle aus menschlichem Blut gewonnenen Materialien wurden auf Syphilis, Antikörper gegen HIV-1 und HIV-2, Hepatitis-B-Oberflächenantigen und Hepatitis-C-Antikörper untersucht und negativ befundet.

Bestimmten Komponenten wurde Natriumazid (<0,1 g/dl) hinzugefügt. Um die Bildung von explosiven Metallaziden in Blei- und Kupferrohren zu vermeiden, sollten die Reagenzien nur zusammen mit großen Wassermengen in die Kanalisation gespült werden.

Chemilumineszenz-Substrat: Kontamination und direkte Sonneneinstrahlung vermeiden. Siehe Packungsbeilage.

Wasser: Destilliertes oder deionisiertes Wasser verwenden.

Im Lieferumfang enthalten

Die Komponenten sind aufeinander abgestimmt. Die Barcode-Etiketten werden für den Assay benötigt.

FSH Testeinheiten (LFS1)
Jede mit Barcode-Etikette versehene Einheit enthält eine mit monoklonalem Anti-FSH-Mausantikörper beschichtete Kugel. Bei 2–8°C bis zum Ablaufdatum haltbar.
LKFS1: 100 Testeinheiten.
LKFS5: 200 Testeinheiten.

Verpackte Testeinheiten vor dem Öffnen stehen lassen, bis sie Zimmertemperatur erreicht haben. Oben entlang der Kante aufschneiden, ohne den Plastikverschluss zu beschädigen. Verpackungen wieder dicht verschließen, damit der Inhalt trocken bleibt.

FSH- Reagenzbehälter (LFS2)
Mit Barcode. 7,5 ml mit alkalischer Phosphatase (Rinderkalbsdarm) konjugiertes FSH Antikörper (monoklonal, Maus) in Pufferlösung mit Konservierungsmittel. Verschlossen und gekühlt aufbewahren: Bei 2–8°C bis zum Ablaufdatum haltbar. Bei entsprechender Lagerung beträgt die empfohlene Aufbrauchsfrist nach dem Öffnen 30 Tage.
LKFS1: 1 Behälter.
LKFS5: 5 Behälter.

FSH-Kalibratoren (LFSL, LFSH)
Zwei Fläschchen (niedrig und hoch) à 3,0 ml FSH in einer nichthumanen Serum-Matrix (mit Konservierungsmittel). 30 Tage nach dem Öffnen bei 2–8°C haltbar oder 6 Monate bei –20°C (aliquotiert).
LKFS1: 1 Set.
LKFS5: 2 Sets.

Separat erhältliche Testsystem-Komponenten

FSH Verdünnungspuffer (LFSZ)
Zum manuellen Verdünnen der Patientenproben. Ein Fläschchen (25 ml) mit FSH-freier nichthumaner Serum-Matrix, mit Konservierungsmittel. 30 Tage nach dem Öffnen bei 2–8°C oder 6 Monate bei –20°C haltbar.

LSUBX: Chemilumineszenz-Substrat
LPWS2: Pipettenwaschlösung
LKPM: Pipettenreinigungsset
LCHx-y: Halterungen für die Probenschalen (mit Barcodierung)
LSCP: Probenschalen (Einwegartikel)
LSCC: Verschlüsse für die Probenschalen (optional)

CON6: Multikomponentenkontrolle in drei Konzentrationen.

Ebenfalls benötigt
Transferpipetten für die Proben; destilliertes bzw. deionisiertes Wasser; Kontrollen.

Testdurchführung

Für eine optimale Funktion des Gerätes ist unbedingt zu beachten, dass die Wartungen, wie im IMMULITE oder IMMULITE 1000-Handbuch beschrieben, regelmäßig durchgeführt werden.

Das Handbuch für das IMMULITE oder IMMULITE 1000 enthält die A`gen für: Vorbereitung, Geräteeinstellungen, Kalibrierung, Testdurchführung und Qualitätskontrollen.

Überprüfen Sie jedes Testeinheiten auf das Vorhandensein der Polystyrol-Kugel vor dem Einsetzen in das Gerät.

Empfohlenes Kalibrationsintervall: 4 Wochen

Proben zur Qualitätskontrolle: Kontrollen oder Poolseren mit FSH in mindestens zwei Konzentrationen (niedrig und hoch) verwenden.

Referenzwerte

Die Referenzbereiche wurden in einer internationalen Multicenter-Studie mit dem IMMULITE-FSH Assay (mono/poly) bestimmt. Es wurden täglich während des gesamten Ovulationszykluses Proben von offensichtlich gesunden Frauen (Alter

16 – 44 Jahre) entnommen. (Siehe Grafik "Menstruationperiode").

		FSH, mIU/ml	
Ovulationszyklen	*n**	Median	95%-Bereich
Follikelphase	54 (762)	6,2	2,8 – 11,3
Follikelphase, 2, bis 3. Tag	54 (108)	6,6	3,0 – 14,4
Mittelzyklus	54 (54)	13,6	5,8 – 21
Lutealphase	54 (604)	3,4	1,2 – 9,0

*Anzahl der Versuchpersonen (Gesamtsahl der Ergebnisse)

Eine weitere Studie mit dem IMMULITE FSH Assay (mono/poly) ergab für erwachsene Männer und postmenopausale Frauen (unbehandelt) folgende Ergebnisse. Die Werte für Frauen unter Kontrazeptiva und für postmenopausale Frauen unter HRT basieren auf der Korrelation zum DPC Coat-A-Count FSH IRMA.

		FSH, mIU/ml	
Gruppe	*n*	Median	95%-Bereich
Männer	135	3,8	0,7 – 11,1
Frauen			
Postmenopause*	76	90,5	21,7 – 153
Postmenopause (HRT)	16	27	9,7 – 111
Orale Kontrazeptiva	12	1,7	NN – 4,9

*Vorläufig NN: Nicht nachweisbar

Für eine altersabhängige Studie wurde in einer "Wellness"-Klinik im Südwesten der USA mit Hilfe des IMMULITE-FSH (mono/poly) folgende Werte ermittelt.

			FSH, mIU/ml	
Gruppe	Alter (Jahre)	*n*	Median	95%-Bereich
Frauen	Schnur	30	NN	
	0,1 – 3	57	2,3	0,11 – 13
	4 – 9	28	0,8	0,11 – 1,6
Männer	Schnur	37	0,24	NN – 1,2
	0,1 – 3	72	0,6	NN – 5,5
	4 – 9	31	0,23	NN – 1,9
Kombiniert	Schnur	67	0,11	NN – 1,1
	0,1 – 3	129	1,1	NN – 10
	4 – 9	59	0,5	NN – 1,8

NN: Nicht nachweisbar

Diese Grenzwerte sind lediglich als *Richtlinien* aufzufassen. Jedes Labor sollte seine eigenen Referenzbereiche etablieren.

Grenzen der Methode

In bestimmten Fällen der Infertilität kann die Behandlung mit menschlichen Gonadotropinen ein potentielles Problem für die genaue Messung der FSH-Spiegel darstellen. Das zugegebene FSH kann eine FSH-Antikörperbildung im Patienten hervorrufen. Dies kann eine Störung des Assays bewirken.

Wegen der pulsatiler Sekretion des LH können Proben, die am gleichen Tag vom gleichen Patienten kommen, weit innerhalb des Referenzbereiches schwanken. Dies entspricht der physiologischen Variabilität des LH.

EDTA würde die Resultate erheblich verfälschen und sollte daher nicht als Antikoagulans verwerdet werden.

Heterophile Antikörper in Humanseren können mit Immunglobulinen aus den Assaykomponenten reagieren und Interferenzerscheinungen innerhalb des in vitro Immunoassays verursachen. (Clin. Chem. 1988:34:27-33) Proben von Patienten, die häufig mit Tier- bzw. Tierserumprodukten zu tun haben, können die erwähnten Interferenzen verursachen und zu anomalen Resultaten führen. Die verwendeten Reagenzien sind so konzipiert, dass das Risiko einer Interferenz mit den zu messenden Proben minimiert ist. Dennoch können potentiell Interaktionen zwischen seltenen Seren und den Testkomponenten auftreten. Zu diagnostischen Zwecken sollten die mit dem Assay erhaltenen Ergebnisse immer in Kombination mit der klinischen Untersuchung, der Patientenanamnese und anderen Befunden gesehen werden.

Leistungsdaten

Siehe Tabellen und Grafiken mit *repräsentativen* Daten für den Assay. Die Ergebnisse sind als mIU/ml ausgedrückt. (Alle Daten wurden – sofern nicht anders angegeben – aus Serumproben in Röhrchen ohne Gelbarrieren oder gerinnungsfördernde Zusätze gewonnen.)

Messbereich: Bis 170 mIU/ml (8,9 ng/ml) [WHO 2nd IRP 78/549] [Interims-Ersatzcode 94/632].

Analytische Sensitivität: 0,1 mIU/ml

High-Dose-Hook-Effect: Bis 50 000 mIU/ml keiner.

Präzision: Die Proben wurden in 40 Ansätzen in Doppelbestimmung, ingesamt also in 80 Tests, gemessen. (Siehe Tabelle „Precision.")

Linearität: Proben wurden in verschiedenen Verdünnungen getestet. (Repräsentative Daten entnehmen Sie bitte der Tabelle „Linearity".)

Wiederfindung: Die getesteten Proben waren mit drei FSH-Lösungen 1:19 versetzt (150, 600 und 1 800 mIU/ml). (Repräsentative Daten entnehmen Sie bitte der Tabelle „Recovery".)

Spezifität: Hochspezifischer Anti-FSH-Antikörper (siehe Tabelle „Specificity").

Bilirubin: Bilirubin hat in Konzentrationen bis zu 200 mg/l keinen Einfluss auf die Ergebnisse, der größer als die Impräzision des Assays selbst ist.

Hämolyse: Hämoglobin hat in Konzentrationen bis zu 600 mg/dl keinen Einfluss auf die Ergebnisse, der größer als die Impräzision des Assays selbst ist.

Lipämie: Triglyceride haben in Konzentrationen bis zu 3 000 mg/dl keinen Einfluss auf die Ergebnisse, der größer als die Impräzision des Assays selbst ist.

Alternativer Probentyp: Um den Einfluss von alternativen Probentypen zu überprüfen, wurde 24 reiwilligen Blut in Plastik-Röhrchen ohne Zusatz (Becton Dickinson), Heparin-Röhrchen, EDTA-Röhrchen und SST Vacutainer-Röhrchen entnommen. Alle Proben wurden mit der IMMULITE FSH Methode bestimmt.

(Heparin) = 1,10 (Serum) + 0,65 mIU/ml

(SST) = 0,94 (einfachen Röhrchen) + 0,12 mIU/ml

(EDTA) = 0,88 (Serum) + 0,57 mIU/ml

Mittelwerte:
11,7 mIU/ml (Serum)
13,5 mIU/ml (Heparin)
11,5 mIU/ml (SST)
10,8 mIU/ml (EDTA)

EDTA-Plasma ist als Probenart nicht geeignet.

Methodenvergleich: Der IMMULITE FSH mono/mono Assay wurde unter Verwendung von 431 Patientenproben mit DPC's IMMULITE FSH mono/poly verglichen. Konzentrationsbereich ca. 0,7 – 130 mIU/ml. Siehe Grafik.) Durch lineare Regression:

(IML m/m) = 0,90 (IML m/p) + 0,35 mIU/ml
r = 0,990

Mittelwerte:
31,4 mIU/ml (IMMULITE mono/mono)
34,5 mIU/ml (IMMULITE mono/poly)

Anwendungsberatung

Bei Rückfragen wenden Sie sich bitte an Ihre DPC Niederlassung.

Hergestellt von Euro/DPC Ltd. unter dem Qualitätssystem ISO 13485:2003.

Español

FSH

Utilidad del análisis: Para su uso en el diagnóstico *in vitro* con los analizadores IMMULITE e IMMULITE 1000 — para la medición cuantitativa de hormona estimulante del folículo (Follitropina, FSH) en suero, como una ayuda en el diagnóstico y tratamiento de las afecciones pituitarias y gonadales.

Referencia: **LKFS1** (100 tests), **LKFS5** (500 tests)
Código del Test: **FSH**
Código de Color: **Gris claro**

Resumen y Explicación del Test

La hormona estimulante del folículo (Follitropina, FSH) es secretada por las células B de la hipófisis anterior bajo el control de la hormona liberadora de gonadotropinas (Gn-RH) producida en el hipotálamo. La FSH favorece el desarrollo y mantenimiento de los tejidos gonadales, que sintetizan y seccretan las hormonas esteroideas. Los niveles séricos de FSH están controlados en el hipotálamo por un mecanismo de retroacción negativo de las hormonas esteroideas. Aunque la FSH y la LH se requieren para una normal funcionalidad sexual, tanto en hombres como en mujeres, los patrones de

secrección son muy diferentes en los dos sexos.

En las mujeres adultas, la FSH promueve el crecimiento y desarrollo de los folículos ováricos. Durante la ovulación, cuando el folículo es liberado, el folículo, ahora denominado cuerpo lúteo, segrega estradiol y progesterona, quienes controlan los niveles circulantes de la FSH, en el hipotálamo, mediante un mecanismo de retroacción negativo. En la menopausia, donde la función ovárica está disminuida, hay un decaimiento en la secrección de estradiol. Debido a la carencia del mecanismo de reatroacción negativo, al estar los niveles de estradiol disminuidos, los niveles de FSH circulante empiezan a incrementarse considerablemente.

En el hombre adulto, la FSH está asociada con la estimulación y mantenimiento de la espermatogénesis. La Testosterona y el estradiol tienen el papel de generar la señal de retroacción negativa al hipotálamo para controlar la liberación de FSH. La infertilidad en los hombres puede ser debida a un hipogonadismo como resultado de un fallo testicular primario. Cualquiera que sea la etiología, el hipogonadismo tiene un resultado neto del aumento drástico de los niveles séricos de FSH, debido a la ausencia de retroacción negativa.

Principio del análisis

IMMULITE/IMMULITE 1000 FSH es un ensayo inmunométrico con dos sitios de unión, quimioluminiscente en fase sólida.

Ciclos de Incubación: 1 × 30 minutos.

Recogida de la muestra

Se recomienda el uso de una ultracentrífuga para aclarar las muestras lipémicas.

Las muestras hemolizadas podrían indicar una mala manipulación de la muestra antes de ser recibida por el laboratorio; en este caso, los resultados deben interpretarse con precaución.

El plasma con EDTA no deberia ser usado como muestra.

La centrifugacion de las muestras de suero antes de que se forme el coagulo puede ocasionar la presencia de fibrina. Para evitar resultados erroneos debidos a la presencia de fibrina, asegurarse que se ha formado el coagulo completamente antes de centrifugar las muestras. Algunas muestras, particularmente aquellas de pacientes sometidos a terapia anticoagulante, pueden requerir mayor tiempo de coagulacion.

Los tubos para recoger sangre de distintos fabricantes pueden producir valores diferentes, dependiendo del material del tubo y de los aditivos, incluyendo barreras de gel o barreras fisicas, activadores de la coagulación y/o anticoagulantes. El FSH IMMULITE/IMMULITE 1000 no ha sido analizado con todos los distintos tipos de tubos. Para obtener detalles sobre los tipos tubos que se han analizado, consulte la sección de Tipos de Muestras Alternativos.

Volumen requerido: 50 µl de suero. (El recipiente de la muestre debe contener, como mínimo, 100 µl más que el volumen total requerido).

Conservación: 7 días a 2–8°C, o 2 meses a –20°C.

Advertencias y precauciones

Para uso diagnóstico *in vitro*.

Reactivos: Mantener a 2–8°C. Desechar de acuerdo con las normas aplicables.

Siga las precauciones universales y manipule todos los componentes como si fueran capaces de transmitir agentes infecciosos. Los materiales derivados de sangre humana han sido analizados y son negativos para sífilis; para anticuerpos frente al HIV 1 y 2; para el antígeno de superficie de hepatitis B y para los anticuerpos de hepatitis C.

Se ha usado Azida sodica, en concentraciones menores de 0,1 g/dl, como conservante. Para su eliminacion, lavar con grandes cantidades de agua para evitar la constitucion de residuos de azidas metalicas, potencialmente explosivas, en las cañerías de cobre y plomo.

Sustrato quimioluminiscente: evite la contaminación y exposición a la luz directa del sol. (Ver el prospecto.)

Agua: Use agua destilada o desionizada.

Materiales suministrados

Los componentes representan un juego completo. Las etiquetas de código de barras son necesarias para el ensayo.

Unidades de análisis de FSH (LFS1)
Cada unidad etiquetada con código de barras contiene una bola recubierta de anticuerpos monoclonales murinos anti-FSH. Estable a 2–8°C hasta la fecha de caducidad.
LKFS1: 100 unidades.
LKFS5: 500 unidades.

Espere a que las bolsas de las unidades de análisis alcancen la temperatura ambiente antes de abrirlas. Ábralas cortando por el extremo superior, dejando el borde del cierre de cremallera intacto. Vuelva a cerrar las bolsas herméticamente para protegerlas de la humedad.

Vial de reactivo de FSH (LFS2)
Con código de barras. 7,5 ml de fosfatasa alcalina (de intestino de ternera) conjugada con monoclonal murino anti-FSH en solución tampón, con conservante. Guardar tapado y refrigerado: estable a 2–8°C hasta la fecha de caducidad. Se recomienda utilizarlo antes de que pasen 30 días después de abrirlo cuando se guarda según lo indicado.
LKFS1:1 vial. **LKFS5:** 5 viales.

Ajustadores de FSH (LFSL, LFSH)
Dos viales 3,0 ml (bajo y alto) de FSH en un suero matriz no humano, con conservante. Estable a 2–8°C durante 30 días después de abrise, o hasta 6 meses (alicuotados) a –20°C.
LKFS1:1 juego. **LKFS5:** 5 juegos.

Componentes del kit que se suministran por separado

Diluyente para muestras de FSH (LFSZ)
Para la dilución manual de las muestras de los pacientes. Un vial (25 ml) de una matriz de suero no humana en solución tampón, libre de FSH, con conservante. Estable a 2–8°C durante 30 días después de abrise, o hasta 6 meses (alicuotados) a –20°C.

LSUBX: Sustrato quimioluminiscente
LPWS2: Lavado de sonda
LKPM: Kit de limpieza de sonda
LCHx-y: Soportes de recipientes de muestras (con códigos de barras)
LSCP: Recipientes de muestras (desechables)
LSCC: Tapas para los recipientes de muestras (opcionales)

CON6: control multiconstituyente de tres niveles.

También necesarios
Pipetas de transferencia de muestras; agua destilada o desionizada; controles.

Ensayo

Aviso: para obtener el funcionamiento óptimo, es importante realizar todos los procedimientos del mantenimiento general según lo definido en el manual del operador de IMMULITE o IMMULITE 1000.

Ver el Manual del Operador del IMMULITE o IMMULITE 1000 para: preparación, procesamiento, ajuste, procedimientos de ensayo y control de calidad.

Inspeccionar visulamente cada unidad de rección para asegurarse de que hay una bola antes de introducirla en el Sistema.

Intervalo de ajuste recomendado:
4 semanas.

Muestras de Control de calidad: Use controles o pools de muestrasro con dos niveles diferentes, como mínimo, de FSH (bajo y alto).

Valores esperados

Los valores de normalidad de la FSH IMMULITE (mono/poli) fueron obtenidos en un estudio multinacional, con mujeres voluntarias en aprente buen estado de salud, edades comprendidas entre 16 y 44 años y con tomas de sangre diarias hasta completar un ciclo completo ovulatorio. (Véase gráfico "Periodo Menstrual")

Ciclos ovulatorios	*n**	FSH, mIU/ml Mediana	Central 95%
Fase folicular	54 (762)	6,2	2,8 – 11,3
Fase folicular, Días 2 a 3	54 (108)	6,6	3,0 – 14,4
Ciclo medio	54 (54)	13,6	5,8 – 21
Fase lútea	54 (604)	3,4	1,2 – 9,0

*Número de individuos (número total de resultados)

Otro estudio realizado con la FSH IMMULITE (mono/poli) ha establecido los siguientes resultados para hombres adultos y mujeres postmenopaúsicas no tratadas. Los rangos para mujeres tomando anticonceptivos orales y mujeres postmenopaúsicas en tratamiento sustitutivo de estradiol están basadas en la correlación del kit FSH IMMULITE con el kit de DPC Coat-A-Count FSH IRMA.

Grupo	*n*	FSH, mIU/ml Mediana	Central 95%
Hombres	135	3,8	0,7 – 11,1
Mujeres			
Postmenopaúsicas	76	90,5	21,7 – 153
Postmenopaúsicas (ERT)	16	27	9,7 – 111
Anticonceptivos orales	12	1,7	ND – 4,9

*Preliminar ND: no detectable

Un estudio sectorial sobre los valores normales de fertilidad pediátrica llevados a cabo con la FSH IMMULITE (mono/poli) en indiviuos aparentemente sanos en suroeste de los Estados Unidos dió lugar a los siguientes resultados.

Grupo	Edad (años)	*n*	FSH, mIU/ml Mediana	Central 95%
Mujeres	Cordón	30	ND	
	0,1 – 3	57	2,3	0,11 – 13
	4 – 9	28	0,8	0,11 – 1,6
Hombres	Cordón	37	0,24	ND – 1,2
	0,1 – 3	72	0,6	ND – 5,5
	4 – 9	31	0,23	ND – 1,9
Combinado	Cordón	67	0,11	ND – 1,1
	0,1 – 3	129	1,1	ND – 10
	4 – 9	59	0,5	ND – 1,8

ND: no detectable.

Estos límites han de considerarse sólo como una guía. Cada laboratorio deberá establecer sus propios intervalos de referencia.

Limitaciones

En ciertos casos de infertilidad, el tratamiento con gonadotropinas humanas plantea un problema potencial para la medición precisa de los niveles de FSH. La FSH que se administra puede causar que el paciente produzca anticuerpos contra la FSH, los cuales interferirán directamente con el ensayo.

Debido a la secrección pulsátil, muestras de un mismo indiciduo tomadas el mismo día pueden variar considerablemente dentro de los valores de normalidad, lo que refleja una variación fisiológica más que un error en la técnica o en la metodología.

Como EDTA tendría un efecto significativo en los resultados, no debe usarse como anticoagulante.

Los anticuerpos heterofílicos en el suero humano pueden reaccionar con las inmunoglobulinas de los componentes del ensayo provocando interferencias con los inmunoanálisis in vitro. [Ver Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Las muestras de los pacientes que frecuentemente están expuestos a animales o a productos séricos animales pueden presentar este tipo de interferencia que potencialmente ocasione un resultado anómalo. Estos reactivos han sido formulados para minimizar el riesgo de interferencia, no obstante, pueden darse interacciones anómalas entre sueros conflictivos y los componentes del ensayo. Con fines de diagnóstico, los resultados obtenidos con este ensayo siempre deben ser usados en combinación con el examen clínico, la historia médica del paciente y cualquier otro dato clínico relevante.

Características analíticas

Para ver resultados *representativos* de las cualidades del ensayo, consulte las tablas y los gráficos. Los resultados se expresan en mIU/ml. (A no ser que se indique lo contrario, todos los resultados fueron generados en muestras de suero

recogidas en tubos sin geles o activadores de la coagulación).

Intervalo de calibración: hasta 170 mIU/ml (8,9 ng/ml). Estandarizado en términos de la WHO 2° IRP 78/549 [código interino de cambio 94/632].

Sensibilidad: 0,1 mIU/ml.

Efecto de gancho a altas dosis: Ninguno hasta 50 000 mIU/ml.

Precisión: Las muestras fueron analizadas por duplicado en 40 tandas para un total de 80 replicados. (Ver la tabla "Precision.")

Linealidad: las muestras fueron analizadas con varias diluciones. (Véase la tabla " Linearity " para resultados representativos).

Recuperación: Se han analizado las muestras cargadas 1 a 19 con tres soluciones de FSH (150, 600 y 1 800 mIU/ml). (Ver la tabla "Recovery" para resultados representativos).

Especificidad: El anticuerpo es altamente específico para FSH. (Véase la tabla "Specificity").

Bilirrubina: La presencia de bilirrubina, en concentraciones hasta 200 mg/l, no tienen ningún efecto sobre los resultados en términos de precisión.

Hemólisis: La presencia de hemoglobina, en concentraciones hasta 600 mg/dl, no tienen ningún efecto sobre los resultados en términos de precisión.

Lipemia: La presencia de triglicéridos en concentraciones hasta 3 000 mg/dl no tiene efecto alguno en los resultados, en lo correspondiente a la precisión del ensayo.

Tipo de Muestra Alternativa: Para evaluar el efecto de los tipos de muestra alternativos, se extrajo sangre a 24 voluntarios en tubos de plástico Becton Dikinson secos, con heparina, con EDTA y tubos SST vacutainer. Todas las muestras fueron analizadas con el ensayo de FSH IMMULITE.

(Heparina) = 1,10 (Suero) + 0,65 mIU/ml

(SST) = 0,94 (tubos simples) + 0,12 mIU/ml

(EDTA) = 0,88 (Suero) + 0,57 mIU/ml

Medias:
11,7 mIU/ml (Suero)
13,5 mIU/ml (Heparina)
11,5 mIU/ml (SST)
10,8 mIU/ml (EDTA)

El plasma con EDTA no deberia ser usado como muestra.

Comparación de los métodos: El procedimiento de IMMULITE FSH mono/mono ensayo se ha comparado con el IMMULITE FSH mono/poli de DPC en 431 muestras de pacientes. (Intervalo de concentración: aproximadamente 0,7 a 130 mIU/ml. Ver la gráfico). Por regresión lineal:

(IML m/m) = 0,90 (IML m/p) + 0,35 mIU/ml
r = 0,990

Medias:
31,4 mIU/ml (IMMULITE mono/mono)
34,5 mIU/ml (IMMULITE mono/poli)

Asistencia técnica

Contácte con su Distribuidor Nacional.

Fabricado por EURO/DPC Ltd. bajo un Sistema de Calidad acorde con la ISO 13485:2003.

Français

IMMULITE FSH

Domaine d'utilisation : Pour le dosage quantitatif de l'hormone folliculostimulante (FSH) dans le sérum. Ce test est réservé à un usage diagnostic *in vitro* avec l'Analyseur IMMULITE ou de IMMULITE 1000 et constitue une aide au diagnostic et au traitement des troubles hypophysaires et gonadiques.

Ce réactif est enregistré auprès de l'AFSSAPS.

Référence catalogue : **LKFS1** (100 tests), **LKFS5** (500 tests)

Code produit : **FSH.**
Code couleur : **gris clair.**

Introduction

L'hormone folliculostimulante (FSH) est sécrétée par les cellules β de l'antéhypophyse sous le rétrocontrôle de l'hormone gonadotrophine releasing (GnRH) produite par l'hypothalamus. La FSH facilite le développement et le fonctionnement du tissu gonadique, lequel synthétise et sécrète les hormones stéroïdiennes. Le taux circulant de FSH

est régulé par un rétrocontrôle négatif sur l'hypothalamus déclenché par les hormones stéroïdiennes. La FSH et la LH sont nécessaires au fonctionnement sexuel normal à la fois chez l'homme et chez la femme, mais les modalités sécrétoires sont différentes suivant le sexe.

Chez la femme mature, la FSH est à l'origine du développement des follicules ovariens. Pendant l'ovulation, le follicule est rompu, il évolue vers le corps jaune en sécrétant l'estradiol et la progestérone lesquels contrôlent le taux circulant de FSH par un rétrocontrôle négatif sur l'hypothalamus. Au cours de la ménopause, il y a diminution de la fonction ovarienne, il en résulte une diminution de la sécrétion d'estradiol. Une baisse du rétrocontrôle négatif initié par la diminution de la concentration d'estradiol entraîne une augmentation significative du taux de FSH circulant.

Chez l'homme mature, la FSH est associée à la stimulation et au maintien de la spermatogenèse. La testostérone et l'estradiol sont à l'origine du rétrocontrôle négatif sur l'hypothalamus et contrôlent ainsi la sécrétion de FSH. La stérilité masculine peut être due à un hypogonadisme lié à une insuffisance testiculaire. L'insuffisance testiculaire peut être liée soit à une insuffisance fonctionnelle acquise ou être le résultat d'une infection microbienne. Quelle que soit son étiologie, l'insuffisance testiculaire a une incidence très nette sur les taux circulants de FSH qui sont augmentés de façon spectaculaire en raison de l'absence de rétrocontrôle négatif.

Principe du test

IMMULITE/IMMULITE 1000 FSH est un dosage chimiluminescent immunométrique, en deux étapes, en phase solide.

Cycles d'incubation : 1 × 30 minutes.

Recueil des échantillons

Il est recommandé de clarifier les échantillons hyperlipémiques par ultracentrifugation.

Des échantillons hémolysés peuvent être révélateurs d'une préparation inadéquate du prélèvement avant son envoi au laboratoire ; il faudra donc interpréter les résultats avec prudence.

Les echantillons ne doivent pas etre des plasmas EDTA.

La centrifugation des échantillons sériques avant la formation complète du caillot peut entraîner la présence de fibrine. Pour éviter les résultats erronés dûs à la présence de fibrine, s'assurer de la formation complète du caillot avant de centrifuger les échantillons. Certains échantillons, en particulier ceux provenant de patients sous anti-coagulants, peuvent nécessiter un temps plus long pour la formation du caillot.

Des tubes pour prélèvements sanguins provenant de fabricants différents peuvent donner des résultats différents, selon les matériaux et additifs utilisés, y compris gels ou barrières physiques, activateurs de la coagulation et/ou anticoagulants. Le coffret FSH IMMULITE/IMMULITE 1000 n'a pas été testé sur tous les types de tubes possibles. Veuillez consulter le chapitre intitulé Autres Types d'Échantillons pour plus de renseignements sur les tubes qui ont été évalués.

Volume nécessaire : 50 µl sérum. (L'unité-échantillon doit contenir au moins 100 µl de plus que le volume total nécessaire.)

Conditions de conservation : 7 jours à +2°C/+8°C ou 2 mois à –20°C.

Précautions d'emploi

Réservé à un usage diagnostique *in vitro*.

Réactifs : conserver les réactifs à +2°/ +8 °C. Eliminer les déchets conformément à la réglementation en vigueur.

Respecter les précautions d'emploi et manipuler tous les composants du coffret comme des produits potentiellement infectieux. Les réactifs dérivés de produits humains et utilisés dans ce coffret ont subi un test sérologique pour la Syphilis et des tests de dépistage pour les anticorps anti-VIH1 et 2, anti-VHC et pour l'antigène de surface de l'hépatite B, qui se sont tous avérés négatifs.

De l'azide de sodium à des concentrations inférieures à 0,1 g/dl a été ajouté comme conservateur ; lors de l'élimination, l'évacuer avec de grandes quantités d'eau

pour éviter une accumulation d'azides métalliques explosifs dans les canalisations.

Substrat chimiluminescent : éviter les contaminations et l'exposition directe à la lumière solaire (voir la fiche technique).

Eau : utiliser uniquement de l'eau distillée ou désionisée.

Matériel fourni

Les composants de la trousse ne peuvent être utilisés que conjointement. Les étiquettes à l'intérieur du coffret sont nécessaires au dosage.

Tests unitaires FSH (LFS1)
Avec code-barre. Chaque unité-test contient une bille revêtue d'anticorps mononclonal murin anti-FSH. Stable à +2°C/+8°C jusqu'à la date de péremption. **LKFS1 :** 100 unités. **LKFS5 :** 500 unités.

Porter les sachets à température ambiante avant d'ouvrir. Ouvrir le sachet avec des ciseaux en préservant le dispositif de fermeture. Refermer les sachets pour les protéger de l'humidité.

Cartouche à réactif FSH (LFS2)
Avec code-barres. 7,5 ml de phosphatase alcaline (intestins de veau) conjuguée à un anticorps monoclonal murin anti-FSH dans un tampon, avec conservateur. Conserver bouché et réfrigéré : stable à +2°C/+8°C jusqu'à la date de péremption. A utiliser de préférence dans les 30 jours qui suivent l'ouverture, si les recommandations de stockage sont respectées.
LKFS1 : 1 cartouche.
LKFS5 : 5 cartouches.

Ajusteurs FSH (LFSL, LFSH)
2 flacons ("haut" et "bas"), 3 ml chacun, de FSH dans une matrice de sérum non humain, avec conservateur. Stable à +2/ +8 C pendant 30 jours après ouverture, ou 6 mois (aliquoté) à –20 °C.
LKFS1 : 1 jeu. **LKFS5 :** 2 jeux.

Composants du coffret fournis séparément

Diluant échantillon FSH (LFSZ)
Pour la dilution manuelle des échantillons patients. Un flacon contenant 25 ml d'une matrice sérique non-humaine sans FSH avec conservateur. Stable à +2°C/+8°C pendant 30 jours après ouverture ou 6 mois (aliquoté) à –20 °C.

LSUBX : Substrat chimiluminescent
LPWSM : Solution de lavage
LKPM : Coffret de décontamination de l'aiguille de prélèvement
LCHx-y : Supports pour unités échantillons (avec code-barre)
LSCP : unités échantillons (à usage unique)
LSCC : Bouchons pour unités échantillons (optionnel)

CON6 : Contrôle multiparamétrique à trois niveaux

Egalement requis
Pipettes pour le transfert des échantillons ; eau distillée ou désionisée ; contrôles.

Protocole de dosage

Noter que pour des performances optimales, il est important de réaliser toutes les procédures de maintenance de routine selon les instructions du Manuel d'Utilisation de l'IMMULITE ou de l'IMMULITE 1000.

Voir le manuel d'utilisation de l'IMMULITE ou de l'IMMULITE 1000 pour la préparation, le démarrage du système, les ajustements, le dosage et les procédures de contrôle de qualité.

Vérifier visuellement que chaque Unité-Test contient bien une bille avant de la charger dans l'automate.

Intervalle d'ajustement recommandé : 4 semaines.

Echantillons pour le contrôle de qualité : Utiliser des contrôles ou des pools de sérums avec au moins deux niveaux de concentration (faible ou élevé) de FSH.

Valeurs de référence

Les valeurs de référence ont été déterminées en utilisant le test IMMULITE FSH (mono/poly) dans une étude internationale incluant des femmes apparemment en bonne santé (âge : 16 – 44 ans), volontaires pour un prélèvement de sang journalier pendant un cycle ovulatoire complet. (Voir graphique "Cycle menstruel" graphique.)

		FSH, mUI/ml	
Cycle ovulatoire	*n**	Médiane	Centré à 95%
Phase folliculaire	54 (762)	6,2	2,8 – 11,3
Phase folliculaire, Jours 2 à 3	54 (108)	6,6	3,0 – 14,4
Milieu de cycle	54 (54)	13,6	5,8 – 21
Phase lutéale	54 (604)	3,4	1,2 – 9,0

*Nombre de sujets (total des résultats).

Le tableau ci-dessous résume les résultats d'une étude réalisée avec le test IMMULITE FSH (mono/poly) sur des hommes adultes et lds femmes postménonausées non traitées. Les domaines pour les femmes sous contraception orale et les femmes postménopausées sous ERT ont été établis sur la corrélation entre le dosage IMMULITE FSH et le dosage Coat-A-Count FSH IRMA de DPC.

		FSH, mUI/mL	
Groupe	*n*	Médiane	Centré à 95%
Hommes (adultes)	135	3,8	0,7 – 11,1
Femmes (adultes)			
postménauposées *	76	90,5	21,7 – 153
postménauposées (ERT)	16	27	9,7 – 111
Sous contraception orale	12	1,7	ND – 4,9

*Préliminaires
ND: non détectable

Une étude en pédiatrie sur les valeurs de fertilité, réalisée avec le test IMMULITE FSH (mono/poly) dans une clinique du sud ouest des Etats-Unis, a donné les résultats suivants.

			FSH, mUI/mL	
Groupe	Âge (année)	*n*	Médiane	Centré à 95%
Filles	Cordon	30	ND	
	0,1 – 3	57	2,3	0,11 – 13
	4 – 9	28	0,8	0,11 – 1,6
Garçons	Cordon	37	0,24	ND – 1,2
	0,1 – 3	72	0,6	ND – 5,5
	4 – 9	31	0,23	ND – 1,9
Total	Cordon	67	0,11	ND – 1,1
	0,1 – 3	129	1,1	ND – 10
	4 – 9	59	0,5	ND – 1,8

ND: non détectable

Utiliser ces valeurs *à titre indicatif* uniquement. Chaque laboratoire devrait établir ses propres valeurs de référence.

Limites

Dans certains cas de stérilité, le traitement avec des gonadotrophines humaines pose des problèmes pour la mesure du taux exact de FSH. La FSH qui est administrée peut entraîner la production par l'organisme d'anticorps anti-FSH qui interfèrent dans le dosage.

Du fait de la sécrétion pulsatile, des échantillons de sérums prélevés le même jour chez une même patiente, peuvent fluctuer largement à l'intérieur du domaine de référence, reflétant ainsi les variations physiologiques plus qu'une erreur technique ou méthodologique.

L'EDTA étant susceptible d'avoir un impact significatif sur les résultats, il ne devrait pas être utilisé comme anti-coagulant.

Les anticorps hétérophiles du sérum humain peuvent réagir avec les immunoglobulines faisant partie des composants du coffret et interférer avec les immunodosages in vitro. [Voir Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Les échantillons provenant de patients fréquemment exposés aux animaux ou aux produits sériques d'origine animale peuvent présenter ce type d'interférence pouvant potentiellement donner un résultat anormal. Ces réactifs ont été mis au point afin de minimiser le risque d'interférence, cependant des interactions potentielles

entre des rares sérums rares et les composants du test peuvent se produire. Dans un but diagnostique, les résultats obtenus avec ce dosage doivent toujours être utilisés en association avec un examen clinique, l'histoire médicale du patient et d'autres résultats.

Performances du test

Consulter les tableaux et graphiques pour obtenir les données *représentatives* des performances du test. Les résultats sont donnés en mUI/ml. (En l'absence de précision supplémentaire, tous les résultats ont été obtenus sur des échantillons sériques prélevés sur tubes sans gel, ni activateur de la coagulation).

Domaine de mesure : jusqu'à 170 mUI/ml (8,9 ng/ml). Conforme aux normes de la 2ème IRP 78/549 de l'O.M.S. [code de remplacement intermédiaire 94/632].

Sensibilité analytique : 0,1 mUI/ml

Effet-crochet aux doses élevées : aucun jusqu'à 50 000 mUI/ml.

Précision : Les échantillons ont été dosés en double essai lors de 40 séries soit un total de 80 résultats. (Voir le tableau « Precision ».)

Linéarité : les échantillons ont été testés avec des taux de dilution variés. (Voir le tableau " Linearity " pour des données représentatives.)

Récupération : les échantillons testés ont été chargés dans un rapport de 1 à 19 avec trois solutions de FSH (150, 600 et 1 800 mUI/ml). (Voir le tableau " Recovery " pour des données représentatives.)

Spécificité : l'anticorps est hautement spécifique de la FSH (Voir le tableau " Specificity ").

Bilirubine : La présence de bilirubine ne présente aucun effet sur les résultats ni sur la précision du dosage si la concentration ne dépasse pas 200 mg/l.

Hémolyse : La présence d'hémoglobine ne présente aucun effet sur les résultats ni sur la précision du dosage si la concentration ne dépasse pas 600 mg/dl.

Lipémie : La présence de triglycérides jusqu'à une concentration de 3 000 mg/dl n'interfère ni sur la précision du dosage, ni sur les résultats.

Autres types d'échantillons : Afin de déterminer l'incidence d'autres types d'échantillons, des prélèvements sur 24 volontaires ont été effectués sur tubes sériques secs en plastique Becton Dickinson, sur tubes de héparine, sur tubes EDTA et sur tubes vacutainers SST®. Tous les échantillons ont été dosés avec la procédure IMMULITE FSH.

(Héparine) = 1,10 (Sérum) + 0,65 mUI/ml

(SST) = 0,94 (tubes ordinaires) + 0,12 mUI/ml

(EDTA) = 0,88 (Suero) + 0,57 mUI/ml

Moyennes :
11,7 mUI/ml (Sérum)
13,5 mUI/ml (Héparine)
11,5 mUI/ml (SST)
10,8 mUI/ml (EDTA)

Les echantillons ne doivent pas etre des plasmas EDTA.

Comparaison de méthodes : le IMMULITE FSH mono/mono dosage a été comparé à l'IMMULITE FSH mono/poly de DPC sur 431 échantillons (intervalle de concentrations : 0,7 à 130 mUI/ml environ. Voir graphique). Par régression linéaire :

(IML m/m) = 0,90 (IML m/p) + 0,35 mUI/ml
r = 0,990

Moyennes :
31,4 mUI/ml (IMMULITE mono/mono)
34,5 mUI/ml (IMMULITE mono/poly)

Assistance technique

En France distribué par DPC France 90 bd National 92257 La Garenne-Colombes

Fabriqué par EURO/DPC Ltd. dans le cadre d'un Système Qualité enregistré sous ISO 13485:2003.

Italiano

FSH

Uso: Ad uso diagnostico *in vitro* con gli Analizzatori IMMULITE ed IMMULITE 1000 — per per la misurazione quantitativa dell'ormone follicolo stimolante, FSH, nel siero, quale ausilio nella diagnosi e cura dei disturbi ipofisari e gonadici.

Codice:
LKFS1 (100 test), **LKFS5** (500 test)

Codice del Test: **FSH**
Colore: **grigio chiaro**

Riassunto e spiegazione del Test

L'ormone follicolo stimolante (follitropina, FSH) è secreto dalle cellule b dell'ipofisi anteriore sotto il controllo della gonadotropina prodotta dall'ipotalamo. L'FSH facilita lo sviluppo ed il mantenimento dei tessuti gonadici che sintetizzano e secernono gli ormoni steroidei. I livelli di FSH in circolo sono controllati da un meccanismo di feedback negativo sull'ipotalamo da parte degli ormoni steroidei. Benchè l'FSH e l'LH siano richiesti per una funzionalità sessuale normale sia nell'uomo che nella donna, i meccanismi di rilascio sono molto diversi nei due sessi.

Nella donna adulta, l'FSH avvia la crescita e lo sviluppo dei follicoli ovarici. Durante l'ovulazione, quando il follicolo si rompe, il follicolo, ora chiamato corpo luteo, secerne l'estradiolo ed il progesterone, che controllano i livelli circolanti di FSH con un effetto di feedback negativo sull'ipotalamo. In menopausa, con una diminuzione della funzionalità ovarica, si ha una conseguente diminuzione della secrezione di estradiolo. A causa della mancanza di feedback negativo, con una diminuzione dell'estradiolo, i livelli di FSH in circolo aumentano notevolmente.

Nell'uomo adulto, l'FSH è associato alla stimolazione ed al mantenimento della spermatogenesi. Il Testosterone e l'Estradiolo hanno il ruolo di fornire il segnale di feedback negativo all'ipotalamo per il controllo del rilascio di FSH. L'infertilità maschile può essere dovuta all'ipogonadismo quale risultato di anomalie primarie dei testicoli. Le anomalie dei testicoli possono essere un'incapacità di portare a termine la maturazione delle cellule spermatiche o il risultato di danni alle cellule germinali. Qualsiasi sia l'eziologia, le condizioni di ipogonadismo portano ad un drammatico innalzamento dei livelli di FSH in circolo, dovuti alla mancanza di un feedback negativo.

Principio del procedimento

IMMULITE/IMMULITE 1000 FSH è un dosaggio immunometrico in chemiluminescenza in fase solida a doppio sito.

Cicli d'incubazione: 1 × 30 minuti.

Prelievo dei Campioni

Si consiglia l'utilizzo di un'ultracentrifuga per schiarire i campioni lipemici.

I campioni emolizzati posson indicare il trattamento non idoneo del campione prima dell'arrivo al laboratorio; per questo motivo, i risultati devono essere interpretati con prudenza.

I campioni di plasma EDTA non devono essere utilizzati.

La centrifugazione dei campioni del siero prima che la coagulazione sia completa può produrre fibrina. Per evitare risultati errati dovuti alla presenza di fibrina, assicurarsi che il processo di coagulazione sia completo prima di centrifugare i campioni. Alcuni campioni, in modo particolare quelli di pazienti sottoposti a terapia con anticoagulanti, possono richiedere tempi di coagulazione più lunghi.

Provette per il prelievo di sangue di produttori diversi possono dare valori differenti, a seconda dei materiali e degli additivi usati, incluso gel o barriere fisiche, attivatori di coaguli e/o anticoagulanti. L'IMMULITE/IMMULITE 1000 FSH non é stato verificato con tutte le possibili variazioni di tipi di provette. Consultare la sezione riguardante Campioni Alternativi per dettagli sulle provette testate.

Volume richiesto: 50 µL di siero. (Il porta campione deve contenere almeno 100 µL più del volume totale richiesto).

Conservazione: 7 giorni a 2–8°C o 2 mesi a –20°C.

Avvertenze e Precauzioni

Per uso diagnostico *in vitro*.

Reagenti: Conservare i reagenti a 2–8°C. Eliminare in conformità alle leggi pertinenti.

Seguire le precauzioni generali e manipolare tutti i componenti come se fossero potenzialmente infetti. I materiali derivati dal sangue umano sono stati

testati con esito negativo per la sifilide, gli anticorpi anti-HIV 1 e 2, l'Antigene di Superficie dell'Epatite B e gli anticorpi Anti-Epatite C.

E' stata aggiunta Sodio Azide a concentrazioni inferiori a 0,1 g/dL come conservante. Al momento dell'eliminazione, irrorare con molta acqua per evitare la formazione di azidi metalliche potenzialmente esplosive nelle tubature di piombo e di rame.

Substrato Chemiluminescente: Evitare la contaminazione e l'esposizione alla luce solare diretta. (Vedi metodica.)

Acqua: Utilizzare solo acqua distillata o deionizzata.

Materiali Forniti

I componenti sono un gruppo accoppiato. Le etichette del codice a barra sono necessarie per la prova.

Test Unit FSH (LFS1)
Ogni test unit con codice a barra contiene una sferetta coattata con anticorpo monoclonale murino anti-FSH. Stabile a 2–8°C fino alla data di scadenza.
LKFS1: 100 unità. **LKFS5:** 500 unità.

Le buste delle unità di prova devono essere a temperatura ambientale prima di aprire. Aprire tagliando lungo il bordo superiore, lasciando intatto la chiusura ermetica. Risigillare le buste per proteggere contro umidità.

Porta Reagente FSH
Con codice a barre. 7,5 mL di fosfatasi alcalina (intestino di vitello) coniugata con un anticorpo monoclonale murino anti-FSH in un tampone, con conservanti. Conservare nel frigorifero con il coperchio: stabile a 2–8°C fino alla data di scadenza. E' raccomandato utilizzare il prodotto entro 30 giorni dall'apertura quando viene conservato nella maniera indicata.
LKFS1: 1 Porta Reagente.
LKFS5: 5 Porta Reagenti.

Calibratori FSH (LFSL, LFSH)
Due fiale (bassa ed alta), ciascuno con 3 mL di FSH in una matrice di siero non umano, con conservanti. Stabile a 2–8°C per 30 giorni dopo l'apertura, e per 6 mesi (aliquotato) a –20°C.
LKFS1: 1 set. **LKFS5:** 2 set.

I componenti dei kit sono forniti separatamente

Diluente dell'FSH (LFSZ)
Per la diluzione manuale dei campioni dei pazienti. Un flacone contenente 25 mL di matrice di siero non umano privo di FSH, con conservanti. Stabile a 2–8°C per 30 giorni dopo l'apertura, o per 6 mesi (aliquotato) a –20°C.

LSUBX: Substrato Chemiluminescente
LPWS2: Tampone di lavaggio dell'Ago
LKPM: Kit di Pulizia dell'Ago
LCHx-y: Tubi porta campioni (con codice a barre)
LSCP: Porta Campioni (monouso)
LSCC: Coperchi per Porta Campioni (opzionali)

CON6: 3 livelli, controllo multicostituito

Materiali richiesti
Pipette per la dispensazione di campioni; acqua distillata o deionizzata; controlli.

Procedura del Dosaggio

Attenzione: per avere prestazioni ottimali, è importante effettuare le procedure di manutenzione di routine cosiccome definito nel Manuale dell'Operatore dell'IMMULITE o IMMULITE 1000.

Vedi il Manuale dell'Operatore IMMULITE o IMMULITE 1000 per: preparazione, setup, calibrazione, dosaggio e controllo di qualità.

Controllate ogni test unit verificando la presenza della sferetta prima di caricarla sullo strumento.

Intervallo di Calibrazione Consigliato: 4 settimane.

Controllo di Qualità: Utilizzare controlli o pool di sieri con almeno due livelli (alto e basso) di FSH.

Valori Attesi

I range di riferimento sono stati ottenuti utilizzando il kit IMMULITE FSH (mono/poli) all'interno di uno studio multi-nazionale che ha coinvolto donne in apparente buono stato di salute (età: 16–44 anni), che volontariamente si sono sottoposte ad un prelievo di sangue giornaliero lungo un intero ciclo ovulatorio. (Vedere "Il ciclo menstruale" grafica.)

		FSH, mIU/mL	
Cicli Ovulatori	*n**	Mediana	Centrale 95%
Fase follicolare	54 (762)	6,2	2,8 – 11,3
Fase follicolare, Giorni da 2 a 3	54 (108)	6,6	3,0 – 14,4
Ciclo centrale	54 (54)	13,6	5,8 – 21
Fase luteinica	54 (604)	3,4	1,2 – 9,0

*Numero di pazienti (numero totale di risultati)

La tabella sottostante riassume i risultati per pazienti maschi adulti e (non trattati) e per donne postmenopausa ottenuti con con il Kit IMMULITE FSH (mono/poli). I range per donne che utilizzano contraccettivi orali e per donne in postmenopausa su ERT si basano sulla relazione del dosaggio con il kit Coat-A-Count FSH IRMA.

		FSH, mIU/mL	
Gruppo	*n*	Mediana	Centrale 95%
Uomini	135	3,8	0,7 – 11,1
Donne			
post menopausale*	76	90,5	21,7 – 153
post menopausale (ERT)	16	27	9,7 – 111
Contraccettivi orali	12	1,7	ND – 4,9

*Preliminare ND: non determinable

Uno studio inter-disciplinare su valori di fertilità pediatrica effettuato con il kit IMMULITE FSH (mono/poli) presso una Clinica nel Sud-Ovest degli Stati Uniti ha prodotto i seguenti risultati.

			FSH, mIU/mL	
Gruppo	Età (anni)	*n*	Mediana	Centrale 95%
Donne	Cordone	30	ND	
	0,1 – 3	57	2,3	0,11 – 13
	4 – 9	28	0,8	0,11 – 1,6
Uomini	Cordone	37	0,24	ND – 1,2
	0,1 – 3	72	0,6	ND – 5,5
	4 – 9	31	0,23	ND – 1,9
Combinato	Cordone	67	0,11	ND – 1,1
	0,1 – 3	129	1,1	ND – 10
	4 – 9	59	0,5	ND – 1,8

ND: non determinable

Detti valori dovrebbero essere considerati solo come *suggerimento*. Ogni laboratorio dovrebbe stabilire i propri range di riferimento.

Limiti

In alcuni casi di infertilità, la cura con gonadotropine potrebbe causare delle difficoltà nella misurazione accurata dei livelli di FSH. L'FSH somministrato potrebbe far creare nel paziente anticorpi anti-FSH stesso, che influenzerebbero direttamente l'analisi.

A cause delle secrezioni intermittenti, i campioni ottenuti lo stesso giorno dallo stesso paziente possono flutturare in maniera considerevole all'interno del range di riferimento, riflettendo variazioni fisiologiche piuttosto che errori nella tecnica o nella metodologia.

Perchè l'EDTA avrebbe un effetto significativo sui risultati, non dovrebbe essere utilizzato come un anticoagulante.

Gli anticorpi eterofili presenti nel siero umano possono reagire con le immunoglobuline presenti nelle componenti del dosaggio provocando un'interferenza con i dosaggi in vitro. [Vedi Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Campioni di pazienti routinariamente esposti agli animali o a prodotti derivati da siero di animali possono presentare questo tipo di interferenza causa potenziale di risultati anomali. Questi reagenti sono stati formulati per minimizzare il rischio di interferenze, tuttavia, possono verificarsi interazioni potenziali tra sieri rari e componenti del test. A scopo diagnostico, i risultati ottenuti da questo dosaggio devono sempre essere utilizzati unitamente all'esame clinico, all'anamnesi del paziente e ad altre indagini di laboratorio.

Prestazioni del Dosaggio

Vedere le tabelle e le grafiche per i dati *rappresentativi* delle prestazioni della prova. I risultati sono espressi in mIU/mL. (Se non è notato altrimenti, tutti i risultati sono stati generati nei campioni di siero raccolti in tubi senza barriere di gelatina o additivi che promuovono la coagulazione.)

Range di Calibrazione: fino a 170 mIU/mL (8,9 ng/mL). Standardizzata in termini di WHO secondo IRP 78/549 [codice sostitutivo ad interim 94/632].

Sensibilità analitica: 0,1 mIU/mL

Effetto di dosi forti:
Nessun effetto fino a 50 000 mIU/mL.

Precisione: I campioni sono stati dosati in duplicato in 40 sedute per un totale di 80 replicati. (Vedi tabella "Precision".)

Linearità: Sono stati dosati campioni in varie forme diluite. (Vedi la Tabella "Linearity" per dati rappresentantivi.)

Recupero: Sono stati dosati campioni ai quali sono state aggiunte tre soluzioni di FSH (150, 600 e 1 800 mIU/mL) 1:19. (Vedi la Tabella "Recovery" per dati rappresentantivi.)

Specificità: Il dosaggio è estremamente specifico per l'FSH. (Vedi la Tabella "Specificity".

Bilirubina: La presenza di bilirubina in concentrazioni fino a 200 mg/L non ha nessun effetto sui risultati entro il range di precisione del dosaggio.

Emolisi: La presenza di emoglobina in concentrazioni fino a 600 mg/dL non ha nessun effetto sui risultati entro il range di precisione del dosaggio.

Lipemia: La presenza di trigliceridi in concentrazioni fino a 3 000 mg/dL non ha nessun effetto sui risultati entro il range di precisione del dosaggio.

Tipo di Campione Alternativo: Tipi di Campione Alternativi: Per determinare l'effetto di campioni alternativi, è stato prelevato del sangue da 24 volontari in provette semplici di plastica per il siero Becton Dickinson, in provette eparinizzate, EDTA e vacutainer SST®. Tutti i campioni sono stati dosati con il dosaggio IMMULITE FSH.

(Eparina) = 1,10 (Siero) + 0,65 mIU/mL

(SST) = 0,94 (tubi semplici) + 0,12 mIU/mL

(EDTA) = 0,88 (Siero)) + 0,57 mIU/mL

Valore medio:
11,7 mIU/mL (Siero)
13,5 mIU/mL (Eparina)
11,5 mIU/mL (SST)
10,8 mIU/mL (EDTA)

I campioni di plasma EDTA non devono essere utilizzati.

Comparazione di Metodi: La IMMULITE FSH mono/mono prova è stata paragonata al IMMULITE FSH mono/poli della DPC in 431 campioni. (Gamma di concentrazione: da 0,7 fino a 130 mIU/mL. Vedi grafico.) Mediante regressione lineare:

(IML m/m) = 0,90 (IML m/p) + 0,35 mIU/mL
r = 0,990

Valore medio:
31,4 mIU/mL (IMMULITE mono/mono)
34,5 mIU/mL (IMMULITE mono/poli)

Assistenza Tecnica

All'estero: Si prega di contattare il proprio Distributore DPC Nazionale.

Prodotto dalla EURO/DPC Ltd. nell'ambito di un Sistema di Qualità Certificato ISO 13485:2003.

Português

FSH

Utilização: Para a medição quantitativa de FSH (Hormona folículo-estimulante) no soro, em diagnósticos in vitro com o Analisador IMMULITE ou IMMULITE 1000, para o diagnóstico *in vitro* e tratamento de doenças da pituitária e gónadas.

Números de catálogo: **LKFS1** (100 testes), **LKFS5** (500 testes)

Código do teste: **FSH**.
Cor: **Cinzento claro**

Sumário e explicação do teste

A hormona folículo-estimulante (FSH – follicle stimulating hormone) é segregada pelas células da pituitária anterior sob controlo da hormona libertadora da gonadotrofina (GRH – gonadotropin releasing hormone) produzida no hipotálamo. O FSH facilita o desenvolvimento e manutenção dos tecidos da gónada, que sintetizam e segregam hormonas esteróides. Os valores de FSH circulante são controlados por mecanismos de "feed-back" negativo, no hipotálamo, por hormonas esteróides. Embora o FSH e LH sejam essenciais para o funcionamento sexual normal nos homens e nas mulheres, o padrão de secreção é muito diferente para os dois sexos.

Na mulher adulta, o FSH inicia o crescimento e desenvolvimento do folículo do ovário. Durante a ovulação, o folículo sofre ruptura, passando a chamar-se

corpus luteum, e segrega estradiol e progesterona, que controlam os níveis de FSH por "feed-back" negativo no hipotálamo. Na menopausa, com diminuição do funcionamento do ovário, há um consequente decréscimo da secreção de estradiol. Devido à falta de "feed-back" negativo, com a diminuição de estradiol, os níveis de FSH circulante tornam-se significativamente mais altos.

No homem adulto, o FSH está associado à estimulação e manutenção da espermatogénese. A testosterona e o estradiol têm a função de fornecer o sinal de "feed-back" negativo ao hipotálamo, controlando os valores de FSH circulante. A infertilidade nos homens pode dever-se ao hipogonadismo como resultado de uma deficiência testicular primária. Uma deficiência testicular pode dever-se a uma disfunção na maturação ou a danos das células germinativas. Apesar da etiologia, as condições do hipogonadismo têm como resultado final o aumento dramático dos níveis de FSH circulante, devido à falta do efeito de "feed-back" negativo.

Princípio do procedimento

A FSH IMMULITE/IMMULITE 1000 é um ensaio imunométrico em fase sólida quimioluminescente de duas voltas.

Ciclos de incubação: 1 × 30 minutos.

Colheita

Recomenda-se o uso de uma ultra centrífuga para clarear amostras lipémicas.

Amostras hemolisadas podem indicar tratamento incorrecto de uma amostra antes do envio para o laboratório; portanto os resultados devem ser interpretados com cuidado.

O plasma EDTA não deve ser usado como um tipo de amostra.

A centrifugação de amostras de soro antes da formação completa do coágulo pode resultar na presença de fibrina. Para prevenir resultados errados devido à presença de fibrina, certifique-se que a formação do coágulo foi completa antes da centrifugação das amostras. Algumas amostras, em especial as de doentes que recebem terapia anticoagulante podem requerer um maior tempo de formação do coágulo.

Os tubos para colheita sanguínea de diferentes fabricantes, podem originar diferentes valores, dependendo dos materiais e aditivos, incluíndo gel ou barreiras físicas, activadores do coágulo e/ou anti coagulantes. IMMULITE / IMMULITE 1000 FSH não foram ainda testados com todas as possíveis variações originadas pelos tipos de tubos. Consultar a secção Tipos de Amostras Alternativas para obter detalhes sobre os tubos que foram testados.

Volume de amostra: 50 µL soro. (El recipiente de la muestre debe contener, como mínimo, 100 µl más que el volumen total requerido).

Estabilidade: 7 dias a 2–8°C, ou 2 meses a –20°C.

Precauções

Para uso de diagnóstico in vitro.

Reagentes: Manter a 2–8°C. Elimine de acordo com as normas aplicadas.

Manipule com as devidas precauções todos os materiais capazes de transmitir doenças infecciosas. As matérias primas obtidas de soro humano foram testadas, dando resultados negativos para a sífilis, para os anticorpos do vírus da imunodeficiência humana (HIV) 1 e 2; para o antigénio de superfície da hepatite B (HBsAg) e para os anticorpos do vírus da hepatite C.

Azida de sódio foi adicionada como conservante; para evitar acumulações de azidas metálicas explosivas em canalizações de cobre e alumínio, os reagentes devem ser rejeitados no esgoto apenas se estiverem diluídos e forem lavados com grandes volumes de água.

Substrato quimioluminescente: Evite contaminação e exposição à luz directa (ver bula).

Água: Utilize água destilada ou desionizada.

Materiais fornecidos

Os componentes formam um conjunto uno e indivisível. Os códigos de barras no interior das caixas são necessárias para o ensaio.

Unidades de Teste de FSH (LFS1)
Cada unidade rotulada com código de barras contém uma pérola revestida com anticorpo monoclonal de rato anti-FSH. Estável até a data de validade a 2–8°C.
LKFS1: 100 unidades.
LKFS5: 500 unidades.

Permita que as saquetas de Unidade de Teste fiquem à temperatura ambiente antes de as abrir. Abra cortando pela ranhura superior, mantendo o fecho intacto. Sele novamente as saquetas para proteger contra a humidade.

(Embalagem de reagentes de FSH (LFS2)
Com código de barras. Contém 7,5 mL de fosfatase alcalina (de intestino de vitela) conjugado com anticorpo murino monoclonal anti-FSH tamponizado, com conservante. Armazene tapado e refrigerado: Estável até à data de validade a 2–8°C. Recomenda-se a utilização até 30 dias após aberto quando armazenado de acordo com as indicações.
LKFS1: 1 embalagem.
LKFS5: 5 embalagens.

Ajustes FSH (LFSL, LFSH)
Contém dois frascos (nível alto e baixo), de 3,0 mL cada, com FSH em matriz de soro não-humano, com conservante. Estável, após a abertura, durante 30 dias a 2–8°C, ou por 6 meses (aliquotado) a –20°C.
LKFS1: 1 conjunto. **LKFS5:** 2 conjuntos.

Componentes do kit fornecidos separadamente

Diluente de amostra para FSH (LFSZ)
Para a diluição manual de amostras de doentes. Um frasco com 25 mL de matriz de soro de origem não humana, sem FSH e com conservante. Estável, após a abertura, durante 30 dias a 2–8°C, ou por 6 meses (aliquotado) a –20°C.

LSUBX: Sustrato quimioluminiscente
LPWS2: Lavado de sonda
LKPM: Kit de limpieza de sonda
LCHx-y: Soportes de recipientes de muestras (con códigos de barras)
LSCP: Recipientes de muestras (desechables)
LSCC: Tapas para los recipientes de muestras (opcionales)

CON6: Controlo multiparamétrico de três níveis.

Também necessário :
Pipetas de transferência de amostra; água destilada ou desionizada; controlos.

Procedimento de doseamento

Têr em atenção que para obter um desempenho óptimo, é importante efectuar todos os procedimentos de manutenção de rotina conforme definido no Manual de Operador do IMMULITE ou IMMULITE 1000.

Ver o Manual do Operador do IMMULITE ou IMMULITE 1000 para: preparação, setup, ajustes, procedimento do ensaio e controlo de qualidade.

Confirme a presença da esfera em cada Unidade de Teste antes de a colocar no sistema.

Intervalo entre ajustes aconselhável: 4 semanas.

Amostras de controlo de qualidade: utilize controlos ou "pools" com, pelo menos, dois níveis (alto e baixo) de FSH.

Valores de Referência

Os seguintes valores de referência foram obtidos utilizando o FSH IMMULITE (mono/poli) através de um estudo multi-nacional em mulheres saudáveis (idade: 16–44 anos), as quais se voluntarizaram a uma análise sanguínea diária durante um ciclo ovulatório completo. Veja "Ciclo Menstrual" graph.)

		FSH, mIU/mL	
Ciclos Ovulatórios	*n**	Mediano	Central 95%
Fase folicular	54 (762)	6,2	2,8 – 11,3
Fase folicular, Dias 2 a 3	54 (108)	6,6	3,0 – 14,4
Meio ciclo	54 (54)	13,6	5,8 – 21
Fase luteal	54 (604)	3,4	1,2 – 9,0

*Número de individuos (número total de resultados)

Um outro estudo realizado com o FSH IMMULITE (mono/poli) forneceu os seguintes resultados para homens adultos e mulheres pós-menopáusicas sem terapêutica de substituição. Os valores para mulheres sob contraceptivos orais e mulheres pós-menopáusicas com

terapêutica de substituição são baseados na relação do FSH IMMULITE com o FSH Coat-A-Count IRMA da DPC.

		FSH, mIU/mL	
Sexo	*n*	Mediana	Central 95%
Homens Adultos	135	3,8	0,7 – 11,1
Mulheres Adultas			
Pós-menopausa*	76	90,5	21,7 – 153
Pós-menopausa (TSE)	16	27	9,7 – 111
Anticoncepcionais orais	12	1,7	ND – 4,9

*Preliminar ND: não é detectável

Um estudo cruzado de valores de fertilidade pediátricos realizado com o FSH IMMULITE (mono/poli) numa clínica de *Repouso* no sudoeste dos E.U.A., forneceu os seguintes resultados.

			FSH, mIU/mL	
Sexo	Idade (anos)	*n*	Mediana	Central 95%
Mulheres.	Cordão	30	ND	
	0,1 – 3	57	2,3	0,11 – 13
	4 – 9	28	0,8	0,11 – 1,6
Homens	Cordão	37	0,24	ND – 1,2
	0,1 – 3	72	0,6	ND – 5,5
	4 – 9	31	0,23	ND – 1,9
Combinados	Cordão	67	0,11	ND – 1,1
	0,1 – 3	129	1,1	ND – 10
	4 – 9	59	0,5	ND – 1,8

ND: não é detectável

Considere estes limites apenas como *directrizes*. Cada laboratório deve estabelecer os seus próprios valores de referência.

Limitações

Em certos casos de infertilidade, o tratamento com gonadotrofinas humanas, apresenta um enorme problema para o doseamento exacto dos níveis de FSH. O FSH que é administrado pode provocar a produção de anticorpos anti- FSH no paciente, que irão interferir diretamente com o doseamento.

Derivado a uma secreção pulsátil, amostras do mesmo paciente recolhidas no mesmo dia, podem flutuar amplamente dentro de uma zona de referência reflectindo uma variação fisiológica e não erros na técnica ou na metodologia.

Como EDTA teria um efeito significativo nos resultados, não deve ser usado como anticoagulante.

Os anticorpos heterófilicos no soro humano podem reagir com as imunoglobulinas presentes no ensaio, causando interferência com os imunoensaios in vitro. [Ver Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Amostras de doentes expostas em rotina a produtos ou soros de animais podem demonstrar este tipo de interferência, potencial causador de resultados anómalos. Estes reagentes foram formulados para minimizar o risco de interferência, contudo podem ocorrer potenciais interacções entre soros (raros) e componentes do teste. Para fins de diagnóstico, os resultados obtidos neste ensaio devem ser sempre analisados em combinação com o exame clínico, história de medicação do doente e outros achados que possam correlacionar.

Características do ensaio

Consulte Tabelas e Gráficos para dados *representativos* do desempenho do doseamento. Os resultados são apresentados em mIU/mL. (Salvo referência em contrário, todos os dados provêm de amostras de soro colhidas em tubos sem anticoagulantes, barreiras de gel ou aditivos promotores da coagulação.)

Calibração: Até 170 mIU/mL (8,9 ng/mL). Padronizado de acordo com os termos do WHO 2nd IRP 78/549 [código de substituição interino 94/632].

Sensibilidade Analítica: 0,1 mIU/mL

Efeito Hook de Alta Dose:
Nenhum até 50 000 mIU/mL.

Precisão: Amostras foram ensaiadas em duplicado 40 vezes num total de 80 réplicas. (Consulte a tabela "Precision.")

Linearidade: As amostras foram doseadas sob vários níveis de diluição. (Ver a tabela de "Linearity" para dados representativos.)

Recuperação: As amostras foram adicionadas na relação de 1 para 19 com três soluções FSH (150, 600 e

1 800 mIU/mL) antes do doseamento. (Ver tabela de "Recovery" para dados representativos.)

Especificidade: O doseamento é específico para FSH. (Ver tabela de "Specificity".)

Bilirrubina: A presença de bilirrubina em concentrações até 200 mg/L não tem efeito em resultados, dentro da precisão do ensaio.

Hemolise: A presença de hemoglobina em concentrações até 600 mg/dL não tem efeito em resultados, dentro da precisão do ensaio.

Lipemia: A presença de triglicéridos em concentrações até 3 000 mg/dL não tem efeito nos resultados, dentro da precisão do ensaio.

Tipo de amostra alternativa: Para determinar o efeito de amostras alternativas, foi colhido sangue de 24 voluntários, em tubos lisos de plástico para soro, com EDTA, heparinizados e tubos de vacum SST® da Becton Dickinson. Todas as amostras foram doseadas com o IMMULITE 2000 FSH.

(Heparin) = 1,10 (Soro) + 0,65 mIU/mL

(SST) = 0,94 (tubos simples) + 0,12 mIU/mL

(EDTA) = 0,88 (Suero) + 0,57 mIU/mL

Médias:
11,7 mIU/mL (Soro)
13,5 mIU/mL (Heparin)
11,5 mIU/mL (SST)
10,8 mIU/mL (EDTA)

O plasma EDTA não deve ser usado como um tipo de amostra.

Comparação de métodos: O IMMULITE FSH mono/mono doseamento foi comparado ao IMMULITE FSH mono/poli da DPC em 431 amostras. (Zona de trabalho: aproximadamente 0,7 a 130 mIU/mL. Ver gráfico.) Regressão linear:

(IML m/m) = 0,90 (IML m/p) + 0,35 mIU/mL
r = 0,990

Médias:
31,4 mIU/mL (IMMULITE mono/mono)
34,5 mIU/mL (IMMULITE mono/poli)

Assistência Técnica:

Por favor contacte o seu Distribuidor Nacional.

Fabricado pela EURO/DPC Ltd. de acordo com o Sistema de Qualidade registado segundo a norma ISO 13485:2003.

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Particularly preferred binding agents are monoclonal antibodies.

The contemporaneous tests of the invention can be conducted repeatedly, generally at an interval of at least a week, to monitor the effectiveness of a course of HRT.

The sample tested in the contemporaneous assays may be a sample of any suitable body fluid from the subject, such as blood, serum, plasma, sweat, tears, crevicular fluid and the like. Most conveniently the sample is a sample of urine.

Although FSH is the preferred analyte for use in accordance with the invention, other members of the gonadotrophin family can be used. These include human chorionic gonadotrophin (hCG), luteinizing hormone (LH) and thyroid stimulating hormone (TSH). All of these gonadotrophins are glycopeptides. Their principal structure comprises two peptide chains. One peptide chain, known as the alpha chain, is common to all members of the family. The other peptide change, known as the beta chain, differs in each molecule. In addition, each molecule contains glycoprotein side chains. The detailed structure of these molecules is not completely understood. However it is believed that variations in the composition of the glycoprotein side chains give rise to different forms ("glycoforms") of each molecule. Those skilled in the art will appreciate that differences in the chemical properties of the glycoprotein side chains may also influence the physical properties (e.g. charge) of the overall molecule, such that different glycoforms may also constitute different isoforms. Thus, in the case of FSH for example, on present scientific knowledge it is believed that the alpha and beta peptide chains are the same in all FSH forms, but subtle differences occur in the glycoprotein side

chains. It is believed that the relative proportions of the forms of FSH existing in the menopause state are different from those in the pre-menopause state.

Prior to this invention it was not appreciated that a specific binding assay could be developed which would differentiate between the FSH forms, to an extent sufficient to enable worthwhile detection of a menopausal state to be achieved.

In a preferred embodiment of the invention both assays are of the sandwich format. Each assay therefore requires two specific binding agents (e.g. antibodies), preferably one directed against the alpha chain and the other against the beta chain of the FSH molecule. The antibody pairs must be different. At least one member of each pair, e.g. the anti-beta antibody, must differ from the corresponding antibody in the other pair. However, this alone does not necessarily lead to the desired objective. We have found surprisingly that an enhanced degree of differentiation between pre-menopausal and post-menopausal FSH can be achieved if the members of the second antibody pair are both different from the members of the first antibody pair. Thus, between the two assays, it is preferred that both the capture antibody and the labelled antibody are different. In a preferred embodiment the invention therefore uses two sandwich-format immunoassays for FSH in which the antibodies are directed against the alpha and beta peptide chains of the molecule, but are exhibiting differences in specificity for certain forms of FSH caused by subtle changes in the glycoprotein side chains.

Antibody pairs appropriate for use in the invention can be identified by screening a range of anti-FSH antibody pairs against FSH samples obtained from pre-menopausal and post